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Front page photo: A farmer's buckwheat field near Maribor, Slovenia (photographed by Dr. K. Ikeda, see 9th International Symposium on Buckwheat at Prague, Czech Republic, pp. 135–136)

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Yield stability of Tartary buckwheat genotypes in Nepal

Bal Krishna JOSHI

Agriculture Botany Division, NARC, Khumaltar, PO Box 1135, Kathmandu, Nepal

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Key words: Adaptation, regression, Tartary buckwheat, yield stability

ABSTRACT

Stability analysis is an important step in developing cultivars for a wide range of environments or for a specific location. The yield stability of 17 Tartary buckwheat genotypes evaluated in Nepal during the two crop seasons of 1999 and 2000 were assessed. The regression coefficient (b) and coefficient of determination (r^2) were used for assessing yield stability and adaptability. Three genotypes, GF-212, Sample-6-1 and Sample-7 were identified as superior genotypes, which were well adapted to all environments, stable and had above average yielding ability. Acc-2223 was good for low yielding environments and Acc-2227-1, MY-2-27-1, GF-5234 and Sample-8 for high yielding environments. These 8 genotypes can be taken for further experimentation or for general cultivation in their respective environments. These results are valid for areas with conditions similar to those of the experiment sites. To generalize the conclusions from experimentation, one has to conduct experiments over many seasons and years.

INTRODUCTION

Buckwheat (*Fagopyrum* spp.) is grown from altitudes ranging from 60 to 4200 m over the entire country and year round in Nepal (Baniya et al., 1995). The existence of two cultivated species (*F. esculentum* and *F. tataricum*), wild species and weedy types indicate the richness of the genetic resources of buckwheat in Nepal (Baniya, 1999). It is useful for human and livestock consumption, for soil improvement and is popular among the farmers due to its short growth duration, good performance in both poor and stress environments, and its high nutritious and medicinal value. Nepal is one of the centers of genetic variability for buckwheat and many landraces and wild species exist which have a wide variation in different traits (Baniya et al., 1995; Ohsako et al., 2001). A total of 278 accessions of buckwheat representing 20 different landraces are conserved in the Genetic Seed House, Nepal Agricultural Research Council (NARC), Kathmandu (Upadhyay and Joshi, 2003). The Hill Crops Research Program (HCRP), NARC, Kabre evaluates buckwheat genotypes collected from different parts of country and from abroad. These are commonly evaluated at three sites, Kabre (1760 m), Khumaltar (1350 m) and Jumla (2423 m) over several years and are selected based on the mean value of traits (Baniya, 2001; Biswokarma et al., 2001). Common (*mithe*) buckwheat is grown from the Tarai area to the high hills but Tartary (*tite*) is grown only in the mid and high hills. Tartary buckwheat is a self pollinated crop and produces a higher yield than *mithe*. It is grown as a summer crop in the high hills (2000–4200 m) and as an autumn and spring crop in the mid hills (1000–2000 m). Nepalese *tite* buckwheat has been studied by Biswokarma et al. (2001), Bimb et al. (2001), Baniya et al. (2001), Nakayama et al. (2001), Subedi et al. (2001), Joshi and Bimb (2002).

Evaluation of genotypes for their consistency of performance under different environments is important in plant breeding programs. The occurrence of a large genotype-environment (GE) interaction poses a major problem of relating phenotypic performance to genetic constitution and makes its difficult to decide which genotypes should be selected. It is important to understand the nature of the GE interaction to make evaluation and the ultimately selection of the superior genotypes more efficient. Bilbro and Ray (1976) mentioned that the use of two additional parameters, adaptation and stability, in conjunction with yield would be of significant benefit in the evaluation and characterization of advanced breeding materials. The decision to release a genotype is usually made on the basis of whether the genotype performance was satisfactory in comparison to the performance of one or more standard cultivars over several crop seasons. Stability analysis has been used by many researchers (Bilbro and Ray, 1975; Eberhart and Russell, 1966; Finlay and Wilkinson, 1963; Guimaraes et al., 1998; Joshi et al., 2003) to decide whether the performance of a genotype was satisfactory.

Stability and adaptation studies are useful for releasing a genotype for cultivation under wide as well as specific environments. There are many methods that can be utilized for stability and adaptation study even though such methods have not been used in Nepalese buckwheat research. This paper reports on the results of stability analysis based on the individual mean yield of 17 Tartary buckwheat genotypes grown at two locations for two years.

MATERIALS AND METHODS

The grain yield of 17 Tartary buckwheat genotypes were taken from Biswokarma et al. (2001). These genotypes were tested at two locations, Kabre, Dolakha and Bijyanagar, Jumla over two years (1999 and 2000). Kabre lies in the mid hill district located at an elevation of 1760 m and Bijayangar lies in the high hill district located at 2423 m. These data were used as generated from four different environments (two locations and two years). The experimental procedures are described by Biswokarma et al. (2001).

The environment-wise analysis of variance indicated significant genotypic effects at all sites. Therefore, stability analysis was necessary to aid in the selection of the best genotype. The stability analysis was carried out following the model of Finley and Wilkinson (1963) and as described by Bilbro and Ray (1976). A similar method was used by Joshi et al. (2003) to select rice genotypes. Wang (2001) used interaction diversity index and linear regression index to test for genotype stability and phenotype stability in buckwheat. In brief the arithmetic average of their respective grain yields for a given location was considered as site mean. Regression analyses were used to ascertain the stability and adaptation of the genotypes. The site mean was used as the independent variable and the individual cultivar yield was used as the dependent variable. Based upon the results of regression coefficient (b) and coefficient of determination (r^2), adaptation and stability of each genotype were interpreted respectively. If the regression coefficient was not significantly different from 1.0, the genotype was considered to be adapted to all environments, if the regression coefficient was significantly larger than 1.0, the cultivar was considered to be better adapted to high yielding environments, if the regression coefficient was significantly smaller than 1.0, the cultivar was considered to be better adapted to lower yielding environments. A genotype was considered stable unless its r^2 value was significantly smaller than that of the standard genotype (Bilbro and Ray, 1976). The square root of the r^2 was tested to see if it differed significantly from that of the standard cultivar. A buckwheat genotype, Local *tite* was used as the standard cultivar. The statistical procedures were followed as described by Steel and Torrie (1980). Computer software, MINITAB and MS Excel were used to analyze the data.

RESULTS AND DISCUSSION

Stability parameters and mean yields are presented in Table 1. All 17 genotypes are labeled as A, H or L, which indicates adaptation to all, high yielding or low yielding environments, respectively, based on the regression coefficient. An ideal genotype is defined here as adapted to all environments, stable and above average in yielding ability. Eberhart and Russel (1966) used the mean square

Table 1. Stability parameters and adaptation characteristics of Tartary buckwheat genotypes†.

SN	Genotype	b	r^2, %	Yield, kg/ha	Adaptation
1	Acc-2223	–0.006C	0.02D	1080.50	L
2	Acc-2227	1.01	80.7	963.50	A
3	Acc-2227-1	2.20C	84.1	1170.50	H
4	Acc-2230	0.838	45.1	908.50	A
5	Acc-2234	–0.059C	3.5D	884.25	L
6	Acc-481-1	0.767	72.1	853.75	A
7	GF-212	0.33	10.5D	1009.50	A
8	GF-216	0.835	49.1	934.25	A
9	GF-5234	1.79C	84.4	1036.00	H
10	Kabre *tite*	0.06C	0.2D	809.25	L
11	Local *tite*	–0.302C	95.6	850.50	L
12	MY-2-27-1	2.075C	96.2	1267.25	H
13	Sample-5-1	1.412	80	978.25	A
14	Sample-6-1	1.116	44.7	1004.25	A
15	Sample-7	1.524	73.2	1041.25	A
16	Sample-8	1.724C	97.2	1072.25	H
17	Sample-9-7	1.579C	96.2	790.00	H

†b, Regression coefficient. r^2, coefficient of determination. A, H, L, adapted to all, high yielding and low yielding environments, respectively. C, Significantly different from 1. D, Significantly smaller than that of the standard genotype.

deviations from the regression (s^2_d) for defining a stable genotype. Here the coefficient of determination was used instead of s^2_d, because, individual replicated data, which is necessary to estimate s^2_d, were not available. A wide variation was found in the regression coefficients, coefficients of determination and yield among these genotypes. The regression coefficient ranged from –0.302 to 2.2 and r^2 ranged from 0.02 to 97.2%. The population mean yield was 979.63 kg/ha with a minimum of 790 kg/ha and a maximum of 1267.25 kg/ha. The coefficient of determination of some buckwheat genotypes was very small. This was possibly due to testing the genotypes in only a few environments or evaluating in quite different locations e.g. Jumla which is a high hill district and Dolakha, a mid hill district. For coordinated trials, more or less similar environments should be selected and the number of locations should be increased. Testing genotypes at more locations is considered to be more important than testing over more years for stability studies (Saeed et al., 1984).

Nine genotypes produced a lower yield than average and eight genotypes produced higher than the average. Nine genotypes have a regression coefficient values that was significantly different from 1 and four genotypes had a r^2 value which was significantly smaller than that of the standard genotype, Local *tite*. Three genotypes, GF-5234, Sample-6-1 and Sample-7 were identified as superior genotypes because of adaptation to all environments, and a stable and above average yielding ability. Eight genotypes were found to be adapted to all environments, four genotypes were adapted to low yielding environments and five genotypes to high yielding environments. A breeder can select genotypes for two proposes, one for cultivation over a wide area and secondly for site-specific production. Both of these purposes can be met from these genotypes. The productivity of all of the genotypes was higher than the average national buckwheat productivity (540 kg/ha).

The regression lines of the four genotypes Acc-2227, Kabre *tite*, MY-2-27-1 and Local *tite* are presented in Fig. 1. These four genotypes are typical examples with respect to stability and adaptability (see Table 1). Acc-2227 was stable and adapted to all environments. Kabre *tite* was unstable and adapted to low yielding environments. MY-2-27-1 was stable and adapted to high yielding environments. Local *tite* was found to be stable and adapted to low yielding environments. MY-2-27-1 was the highest yielding Tartary genotype with a regression coefficient of 2.075.

A scatter plot of all the genotypes considering their regression coefficient and genotype mean yield is shown in Fig. 2. This plot helps in selecting genotypes easily for all, low yielding and high yielding environments. Any genotypes falling in the upper-right guardant have above average yields and their regression coefficients are greater than one. Their genotypes are highly responsive to the environment i.e. they are good for high yielding environments. Genotypes in the lower-left guardant respond less to changes in the environment, as their regression coefficients are less than 1 with yields below average. They are less adapted to favorable environments and differ in adaptation to unfavorable environments. Growers can grow genotypes falling in the upper right guardant if the environment is favorable and can select genotypes falling in lower-left guardant if the environment is poor. Genotypes such as Sample-6-1, and Sample-7 have regression

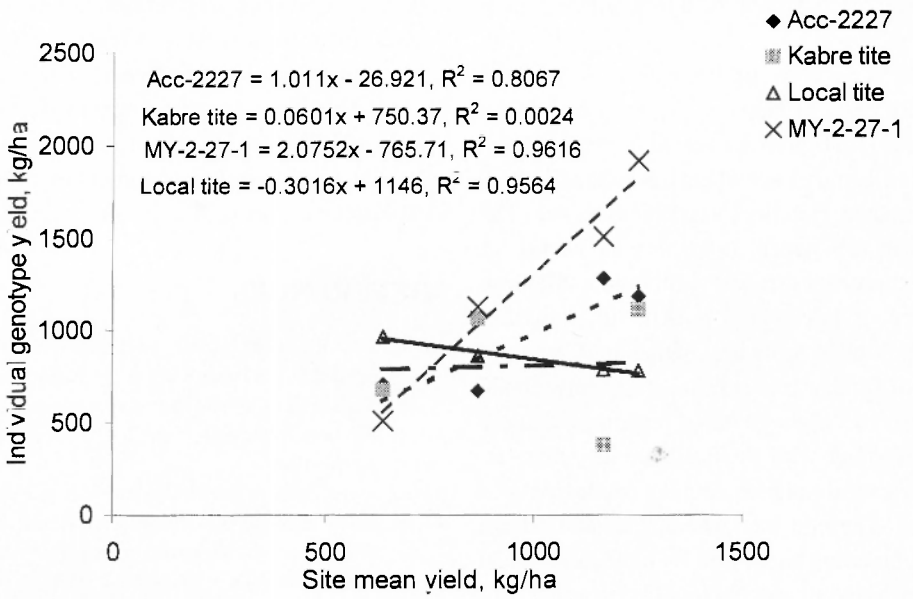

Fig. 1. Regression lines showing the relationship between individual genotype yield and site mean yield of 4 Tartary buckwheat genotypes.

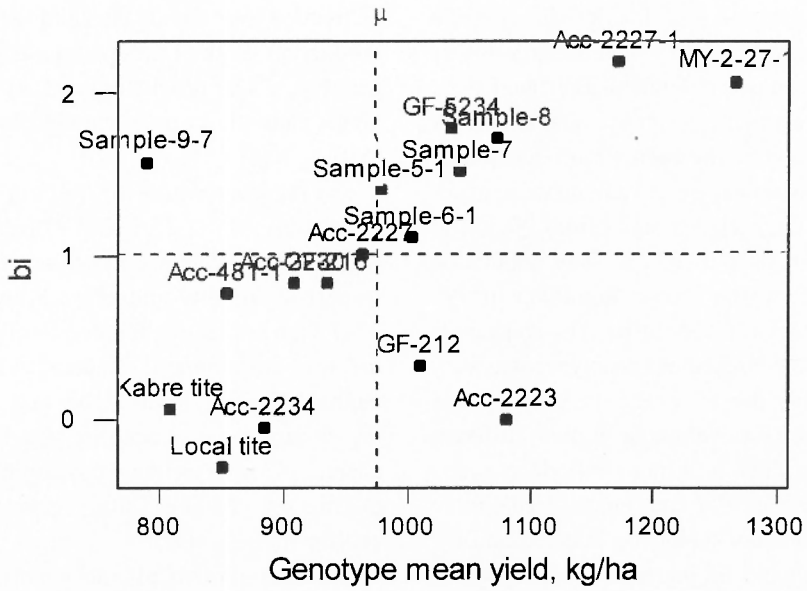

Fig. 2. The relationship of genotype adaptation (bi, regression coefficient) and genotype mean yield of 17 tartary buckwheat genotypes (μ, population mean).

coefficients near to 1.0 with mean yields above average and are considered to be stable. The highly desirable attributes of a genotype to be released for diverse growing conditions are those which displayed strong response in those environments in which site mean yields were high, and also yielded relatively well in trials with a low site mean yields. MY-2-27-1 is a typical genotype, which is very sensitive to changes in the environments, producing very low grain yield in low yielding environment, however the yield increases greatly as the environments improve. Under the most favorable conditions, it is one of the highest-yielding genotypes. This genotype can therefore be described as being specifically adapted to high-yielding environments and is characterized by a regression coefficient of 2.075 which is significantly greater than 1.0.

More than 600 accessions of buckwheat have been studied in different parts of Nepal (Upadhyay and Joshi, 20003; Baniya, 2001). High yield variation between and within common and Tartary buckwheat has been observed. However, performance stability was not assessed. The stability findings in this paper could be very useful for further experimentation or general cultivation. Manipulating the genetics of buckwheat is difficult, however, different biotechnological tools are available to manipulate common and Tartary buckwheat genetically (Joshi and Bimb, 2001). The high yielding response of these Tartary genotypes along with their adaptability and stability may be very useful in increasing the productivity as well as improving common buckwheat. Earlier findings and conventional breeding supported by biotechnological tools should be included in any buckwheat breeding program.

Among the 17 Tartary buckwheat genotypes, Acc-2223 is good for low yielding environments, GF-5234, Acc-2227-1, MY-2-27-1 and Sample-8 for high yielding environments and Sample-6-1, Sample-7 and GF-212 for all environments. These 8 genotypes should be considered for further experimentation, for inclusion in breeding program or for general cultivation in their respective environments. Since the genotypes varied in response to the diverse growing conditions of Nepal, future recommendation for cultivar release should take into account these findings. This experiment was conducted at two locations over two years. In a crop improvement program it is better to relate adaptability and stability performance with the characteristics of the genotypes. The results are valid for the areas with conditions similar to those of the experimental sites. The growth and behavior of the genotypes differ from season to season and from year to year as a result of seasonal and yearly variation in weather patterns. Therefore one can't generalize the conclusions on yield stability for other seasons or years. To generalize the conclusions from experimentation one has to conduct experiments over many seasons and years.

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Morphological and genetic characteristics of *Fagopyrum homotropicum* plants with red-winged seeds discovered in Changbo village, Batang district of Sichuan province in China

Mitsuyuki TOMIYOSHI and Ohmi OHNISHI

Plant Germ-plasm Institute, Graduate School of Agriculture, Kyoto University, Nakajo 1, Mozume-cho, Mukoh 617-0001, Japan

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Key words: Allozymes, Batang district, Sichuan province, *F. homotropicum*, Morphological analysis, red-winged seed

ABSTRACT

Newly discovered plants with red-winged seeds of *Fagopyrum homotropicum* Ohnishi from Batang district of Sichuan province were investigated for their chromosome number, morphological characteristics and allozymes. The red-winged seed plants were found to be diploid. The red-winged type was morphologically quite different from the normal diploid type of *F. homotropicum*. Significant differences were observed for 10 out of 14 characters investigated. Allozyme variation at 15 loci for 10 enzymes were investigated for three diploid and three tetraploid populations. It was found that the diploid Changbo-2 population (2xC0130), which included the red-winged seed plants had five unique alleles at four loci, and showed the highest average heterozygosity (H_e=0.131) in all the populations assayed. The plants with red-winged seeds might have occurred from normal type by mutations.

INTRODUCTION

Fagopyrum homotropicum is a wild self-fertilizing species that was found to be closely related to wild common buckwheat, *F. esculentum* ssp. *ancestrale* (Ohnishi and Matsuoka, 1996; Yasui and Ohnishi, 1998a, b). *F. homotropicum* was first discovered in Yongsheng district of Yunnan province of China in 1992 (Ohnishi, 1995, 1998). Since the discovery of *F. homotropicum*, several researchers have attempted to transfer the self compatible property of *F. homotropicum* to common buckwheat in an attempt to stabilize and increase yield (Campbell, 1995; Hirose et al., 1995; Woo et al., 1995, 2001) and these attempts have been quite successful.

Taxonomic studies of the genus *Fagopyrum* suggested that *F. homotropicum* probably differentiated from *F. esculentum* ssp. *ancestrale* (Ohnishi and Matsuoka, 1996; Yasui and Ohnishi, 1998a). Ohnishi and Asano (1999) showed that both diploid (2n=16) and tetraploid (2n=32) cytotypes are distributed in Yunnan and Sichuan provinces of China, and that the distribution of the tetraploid cytotype was limited to the northwestern part of Yunnan province and the southwestern border of Sichuan province. Populations of *F. homotropicum* from eastern Tibet, which were recently found, were initially assumed to be tetraploid because of their large seeds (Ohnishi and Konishi, 2001).

In 2001, Ohnishi found *F. homotropicum* plants which had red stems, red leaves, pink flowers and red-winged pericarps near the village of Changbo in Batang district during his expedition to the border areas of Sichuan, Yunnan and Tibet (The three river region) (Ohnishi, 2002). This type of plant has never been reported to have been found anywhere else. Judging from its seed size and the geographical distribution of the diploid and tetraploid populations (Fig. 1), the red-winged plants were considered to be tetraploid (Ohnishi, 2002).

In the present study, we attempted to characterize the red-winged seed plants and the population which contained these individuals. We first counted the chromosome number of the red-winged seed plants. Then some morphological differences between the 'red-winged seed' plants (red-winged type) and the 'small seed' plants (normal type), were evaluated by observing and measuring 14 characters. Finally, genetic differences as shown by allozyme variation between diploid populations which included or did not include the red-winged type, was assayed for 15 loci of 10 enzymes.

MATERIALS AND METHODS

Plant materials

F. homotropicum was found growing on rocky barren mountain slopes, and on stony barren roadsides near Changbo village (see Fig. 2), along with wild *F. tataricum* and *F. jinshaense*. Some were found in very large populations, consisting of more than 500 individuals, and others in small populations of less than 50 plants scattered along the roadside. Two large populations, one of them which contained mostly red plants with red-winged seeds (Fig. 2), were chosen as representative populations of near Changbo. More than 300 seeds were randomly collected, 1–5 seeds from each individual, from about 100 individuals. These were recorded as accessions C0129

(Changbo-1) and C0130 (Changbo-2). One more accession (C0131) from the village of Xuebo, which is near Changbo, was also evaluated (see Table 1 and Fig. 1). The seed samples of these accessions were collected by the junior author during his expedition to China in 2001. Both small and large seeds were included in each accession, but the 'red-winged seeds' were included only in C0130 (Fig. 3). Small and large seeds were tentatively considered to be diploid and tetraploid, respectively. The red-winged seeds were treated separately from the small and large seeds in C0130 (see Table 1). Diploid and tetraploid seed samples of each accession were considered to be distinct populations, since *F. homotropicum* is a cleistogamous self-fertilizing species and has little chance of being cross-pollinated.

Chromosome count

Root tips of germinated seeds were used to observe mitotic metaphase chromosomes by using the procedures as described by Ohnishi and Asano (1999). At least five individuals were examined for each seed type. The small seeds, large seeds and red-winged large seeds in each accession (Changbo-1, 2 and Xuebo) were surveyed for chromosome number.

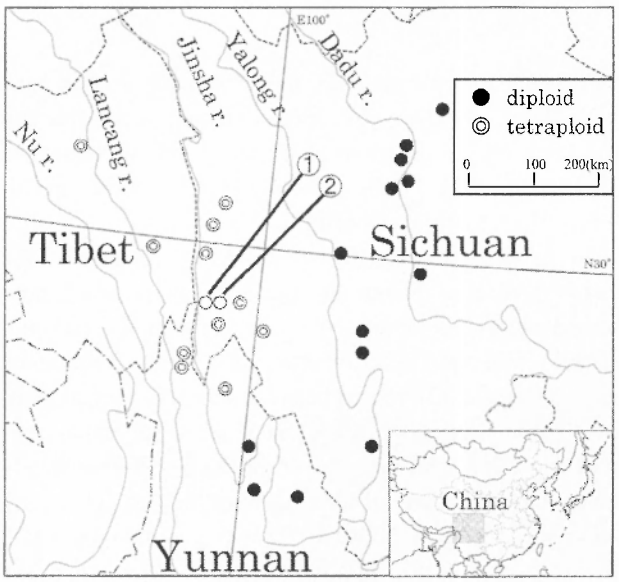

Fig. 1. Location of the accessions studied (① Changbo and ② Xuebo). The dotted area indicates the area of distribution of *F. homotropicum*.

Morphological analyses

Fifteen individuals of the normal small type (2xC0130) and twenty of the red-winged type (rwC0130) were analyzed for morphological characteristics. The plant materials were grown in a greenhouse at the Plant Germplasm Institute, Kyoto University, Mukoh city, Kyoto prefecture, Japan. Fourteen characters listed in Table 2 were scored on each individual following the methods of Yamane and Ohnishi (2003) and Ohsako and Ohnishi (1998). Morphological differences between normal diploid *F. homotropicum* (normal type) and red-winged plants (red winged type) were compared using Microsoft Excel Statistical Analysis System Program (2000) for Windows. Seed weight was measured for 80 seeds of each plant type which had been collected from the natural populations.

Isozyme analyses

Three diploid (2xC0129, 2xC0130 and 2xC0131) and three tetraploid (4xC0129, 4xC0130 and 4xC0131) populations were examined. The normal diploid type and the red-winged type of plants in C0130 were treated as one population (2xC0130) as the red-winged plants were revealed to be diploid by their chromosome number. The method in Ohnishi and Asano (1999) was adopted for electrophoretic analyses of the isozymes. A tris-citrate buffer with EDTA, pH 8.0 was used on dehulled germinated seeds. The enzymes assayed were Alcohol dehydrogenase (ADH, E.C. 1.1.1.1), Diaphorase (DIA, E.C. 1.6.4.3), Glutamate dehydrogenase (GDH, E.C. 1.4.1.2), Glutamate oxaloacetate transaminase (GOT, E.C. 2.6.1.1), Isocitrate dehydrogenase (IDH, E.C. 1.1.1.42), Malate dehydrogenase (MDH, E.C. 1.1.1.37), Phosphoglucomutase (PGM, E.C. 2.7.5.1), Phosphoglucose isomerase (PGI,

Table 1. Samples of *Fagopyrum homotropicum* used in this study

Accession no.	Abbreviation	Seed morphology	Locality			Location in Fig. 1.
			Village	District	Province	
C0129	2xC0129	small	Changbo-1	Batang	Sichuan	①
	4xC0129	large	Changbo-1	Batang	Sichuan	①
C0130	2xC0130*	small	Changbo-2	Batang	Sichuan	①
	rwC0130*	red-winged large	Changbo-2	Batang	Sichuan	①
	4xC0130	large	Changbo-2	Batang	Sichuan	①
C0131	2xC0131	small	Xuebo	Batang	Sichuan	②
	4xC0131	large	Xuebo	Batang	Sichuan	②

*In isozyme analysis, 2xC0130 and rwC0130 treated as one population (2xC0130) on the basis of the chromosome count.

Table 2. Characteristics used in morphological analysis

No.	Morphological characteristics	Unit or Category	Means		Difference*
			normal type	red-winged type	
1	shape of cotyledon	acuminate=0, round=1	0.933	0.950	
2	shape of leaf	hastate=0, intermediate=1, heart=2	0.267	2.000	○
3	number of leaves **	number/individual	2.867	2.550	
4	number of nodes **	number/individual	5.733	4.500	○
5	internodal length ***	cm	1.773	2.570	○
6	vein pubescence (surface side)	none=0, slightly=1, heavily=2	0.533	0.050	
7	vein pubescence (reverse side)	none=0, slightly=1, heavily=2	1.333	0.600	○
8	stem pubescence	none=0, slightly=1, heavily=2	1.200	0.150	○
9	stipule pubescence	none=0, slightly=1, heavily=2	1.467	0.700	○
10	flower color	white=0, pink=1, red=2	0.133	0.750	○
11	anther color	white=0, pink=1, red=2, deep red=3	1.333	3.000	○
12	anthocyanin pigmentation (cotyledon)	none pigmentation=0, slightly=1	0.200	0.850	○
13	anthocyanin pigmentation (leaf)	none pigmentation=0, most heavily=3	1.667	2.450	○
14	seed weight****	g/seed	0.010	0.019	–

* significant at P=0.05
** 3 weeks after sowing
*** length between first and second node from the ground
**** means of 80 seeds

E.C. 5.3.1.9), 6-Phosphogluconate dehydrogenase (6-PGDH, E.C. 1.1.1.44) and Shikimate dehydrogenase (SDH, E.C. 1.1.1.25). The number of individuals examined varied from 18 to 31 (average of 25).

Data analyses

A genotype was assigned for each band morph on the basis of the genotypes of *F. homotropicum* assigned by Ohnishi and Asano (1999), and according to the general agreement of enzyme substructure in higher plants (Gottlieb, 1981). Gene frequency was calculated on the basis of assigned genotypic frequencies. Nei's genetic distance (1987) was calculated for each pair of populations. Based on the genetic distance, a phylogenetic tree was constructed using the neighbor-joining (NJ) method (Saitou and Nei, 1987) and utilizing PHYLIP ver. 3.5 package (Felsenstein, 1993).

RESULTS

Chromosome count

It was verified that the small seeds were diploid (2n=16) and that the large seeds were tetraploid (2n=32), respectively (see Fig. 4). Contrary to our expectation, the red-winged large seeds were revealed to be diploid (2n=16). So each accession was composed of both diploid and tetraploid plants.

Morphological character

The mean and standard error of the 14 characters for each of the two types of plants are shown in Table 2. The following 10 characters showed significant differences between normal and red-winged types: leaf shape, number of nodes, internodal length, vein pubescence on the underside of the blade, stem pubescence, stipule pubescence, flower color, anther color and anthocyanin pigmentation in the cotyledons and leaves (Fig. 5). In addition to these differences the red-winged type also demonstrated a distinct character, a heart shaped leaf. The seed weight of the red winged type was approximately twice that of normal diploid seed type.

Isozyme analyses

In total, 15 loci were analyzed of 10 enzymes. 7 loci (ADH, GDH, IDH, MDH-1, MDH-2, GOT-1 and 6-PGDH-2) were found to be monomorphic in all populations. The allele frequencies at 8 polymorphic loci are shown in Table 3. The diploid Changbo-2 (2xC0130) population had a unique allele at each of three loci (PGM-1, DIA, SDH) and two unique alleles at the PGI locus. The diploid Changbo-1 (2xC0129) population had a unique allele at the MDH-3 and GOT-2 loci. No unique

Table 3. Allele frequency at eight polymorphic loci of *F. homotropicum*

Population	No. of samples	6-PGDH-1		MDH-3			GOT-2			PGI		
		a	b	a	b	c	a	b	c	a	b	c
2xC0129	24	0.329	0.671	0.016	0.953	0.031	0.016	0.453	0.531	0.000	1.000	0.000
2xC0130	31	0.973	0.027	0.044	0.956	0.000	0.000	0.489	0.511	0.188	0.438	0.375
2xC0131	20	0.463	0.537	0.000	1.000	0.000	0.000	0.320	0.680	0.000	1.000	0.000
4xC0129	18	0.500	0.500	0.000	1.000	0.000	0.000	0.500	0.500	0.000	1.000	0.000
4xC0130	31	0.500	0.500	0.000	1.000	0.000	0.000	0.500	0.500	0.000	1.000	0.000
4xC0131	24	0.500	0.500	0.000	1.000	0.000	0.000	0.500	0.500	0.000	1.000	0.000

Population	PGM-1			PGM-2		SDH		DIA				
	a	b	c	a	b	a	b	a	b	c	d	n
2xC0129	0.956	0.044	0.000	0.980	0.020	0.000	1.000	0.077	0.923	0.000	0.000	0.000
2xC0130	0.029	0.000	0.971	1.000	0.000	0.913	0.087	0.650	0.000	0.000	0.050	0.300
2xC0131	0.865	0.135	0.000	0.967	0.033	0.000	1.000	0.000	0.067	0.000	0.933	0.000
4xC0129	0.500	0.500	0.000	0.500	0.500	0.000	1.000	0.000	0.000	0.500	0.500	0.000
4xC0130	0.500	0.500	0.000	0.632	0.368	0.000	1.000	0.167	0.000	0.417	0.417	0.000
4xC0131	0.500	0.500	0.000	0.500	0.500	0.000	1.000	0.000	0.000	0.500	0.500	0.000

allele was found in the diploid Xuebo (2xC0131) population. The diploid Changbo-1 (2xC0129) and diploid Xuebo (2xC0131) populations had similar allele frequencies at seven loci, with the exception of the DIA locus, whereas the diploid Changbo-2 (2xC0130) population showed different allele frequencies when compared to other two diploid populations. Table 4 summarizes the allozyme variation found in the populations. The proportion of polymorphic loci (P) varied from 0.333 (2xC0131) to 0.467 (2xC0130) and the average heterozygosity (H_e) ranged from 0.087 (2xC0129) to 0.131 (2xC0130). The diploid Changbo-2 (2xC0130) population showed the highest variation in the electrophoretic analyses of the isozymes. In the two tetraploid populations (4xC0129 and 4xC0131), heterozygotic bands were found to be fixed at five loci: PGM-2, PGM-1, 6-PGDH-1, GOT-2 and DIA. The same fixed heterozygotic bands were observed in the selfed progeny of these two populations which confirmed that the heterozyygotic bands were due to the double homozygotes *aa*, *bb* at homologous loci in the tetraploids.

Table 4. Average heterozygosity (He), propotion of polymorphic loci (P), and average number of alleles per locus (A) for each population

Population	H_e	P	A
2xC0129	0.087	0.400	1.533
2xC0130	0.131	0.467	1.600
2xC0131	0.090	0.333	1.333
average for 2x	0.103	0.400	1.488
Other plants*	0.074	0.200	1.310

*For other self-fertilizing plant species, after Hamrick and Godt (1989).

Genetic distance and phylogenetic tree

The estimated genetic distance for all pairs of populations is shown in Table 5. The longest distance (0.647) was observed between the diploid Changbo-1 (2xC0129) and Changbo-2 (2xC0130) populations. The diploid Changbo-2 population also had a very long distance from the other five populations. The genetic distance between the tetraploid Changbo-1 (4xC0129) and Xuebo (4xC0131) populations was 0, and the distance between these two and the tetraploid Changbo-2 (4xC0130) was also very small. A phylogenetic tree of these relationships is shown in Fig. 6 where only the diploid Changbo-2 population was found to be distantly related to the other five populations. The three tetraploid populations were found to be clustered together.

DISCUSSION

Distribution of diploid *F. homotropicum*

As shown in Fig. 1, only tetraploid *F. homotropicum* had previously been found in the border area of Sichuan, Yunnan and eastern Tibet (The three river region) (Ohnishi, 2002). By counting the chromosome numbers, however, it was revealed that each accession analyzed in this study consisted of a mixture of both diploid and tetraploid cytotypes of *F. homotropicum* (Fig. 4). In the

Fig. 2

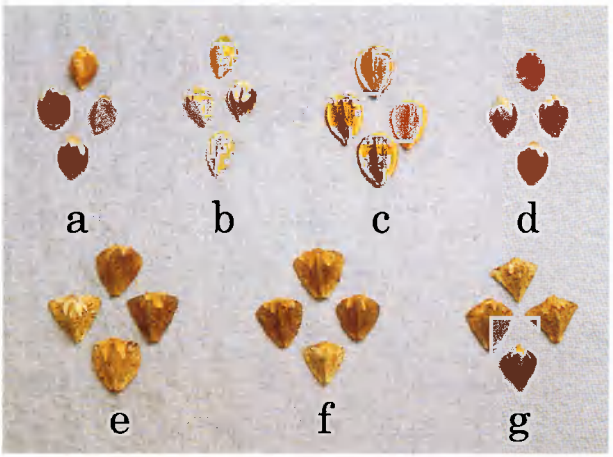

Fig. 3

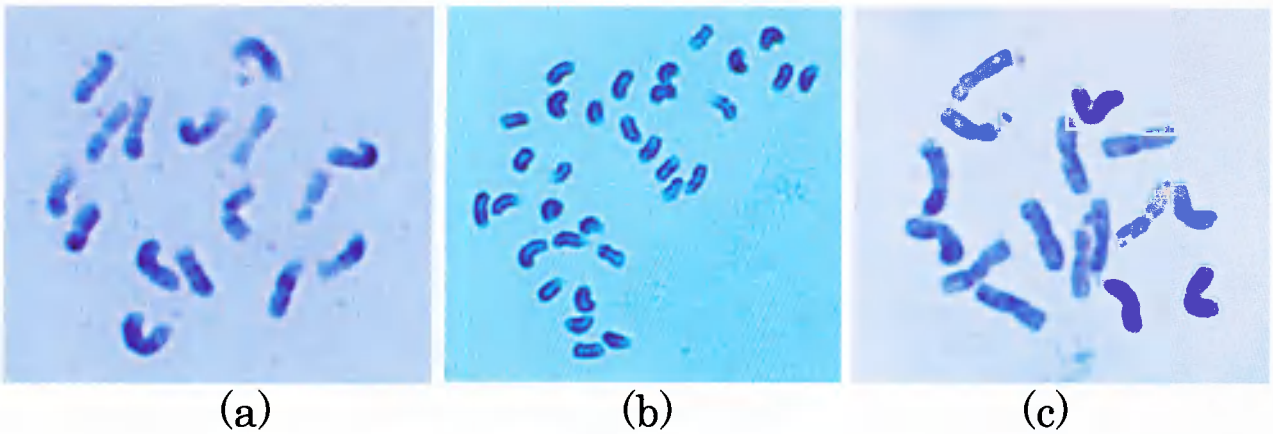

Fig. 4

Fig. 5

Fig. 2. Red-winged seed plants grown in Changbo village, Batang district of Yunnan province.

Fig. 3. Seed morphology of *F. homotropicum*.
a: small seeds in Changbo-1 (2xC0129)
b: small seeds in Changbo-2 (2xC1030)
c: red-winged large seeds in Changbo-2 (rwC0130)
d: small seeds in Xuebo (2xC0131)
e: large seeds in Changbo-1 (4xC0129)
f: large seeds in Changbo-2 (4xC0130)
g: lage seeds in Xuebo (4xC0131)

Fig. 4. Somatic metaphase of *F. homotropicum*.
a: small seeds (2n=2x=16)
b: large seeds (2n=4x=32)
c: red-winged large seeds (2n=2x=16)

Fig. 5. Morphological difference between two types of plants, (a) normal diploid type and (b) red-winged diploid type.
Upper stand: shape of foliage leaves
Middle stand: color of flower and anthers
Lower stand: seeds before shattering

Table 5. Genetic distance based on isozyme variability

	2xC0129	2xC0130	2xC0131	4xC0129	4xC0130
2xC0130	0.647				
2xC0131	0.137	0.596			
4xC0129	0.204	0.645	0.101		
4xC0130	0.168	0.562	0.084	0.007	
4xC0131	0.204	0.645	0.101	0.000	0.007

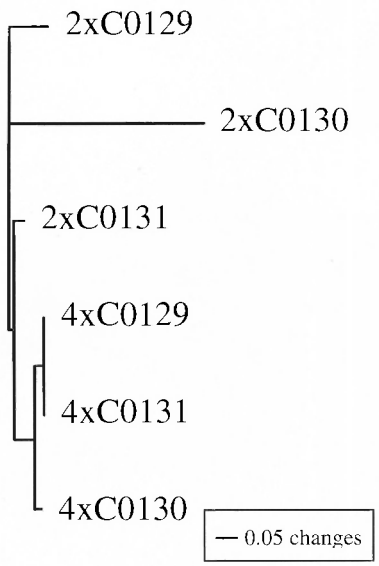

Fig. 6. Phylogenetic tree constructed by neighbour joining method based on genetic distance of allozyme frequencies. 2x= diploid and 4x=tetraploid.

Changbo-2 accession, there were two distinct diploid types (normal and red-winged type). The diploid populations of *F. homotropicum*, therefore, appear to be distributed much wider than previously considered.

Morphological characters of red winged seed plants

The red-winged seed plants were morphologically quite different from the normal diploid type. As shown in Table 2, the anthocyanin pigmentation of the red winged type is intense. Red-winged plants in the natural population in Batang district of Sichuan province had stronger anthocyanin pigmentation than those grown in a greenhouse (see Fig. 2).

The results of the present study invite the question; why do two morphologically distinct types co-exist in diploid Changbo-2 population? When considering the distribution of red-winged seed plants only in Changbo-2, a possible explanation may be that the red-winged type might have occurred from normal type by mutations, and then increased in number in the population. Ohnishi and Asano (1999) stated that morphological differentiation among populations was not clear in *F. homotropicum*. However, the present morphological study has shown that the red-winged seed plants are drastically different in morphology from ordinary diploid plants. They are so different that the red-winged seed plants appear to be a different species. We have to analyze the genetic basis of these morphological differences between the red-winged seed plants and ordinary diploid plants in order to clarify the possible origin of these plants in any local population.

Genetic characteristics of diploid Changbo-2 (2xC0130) population

The allozyme data showed that the diploid Changbo-2 population had five unique alleles at four loci, and had a different allozyme constitution from other populations (Table 3 and Fig. 6). The locus PGI was reported as monomorphic in all *F. homotropicum* populations studied by Ohnishi and Asano (1999), but was found to be polymorphic in the diploid Changbo-2 population (Table 3).

The average heterozygosity of the three diploid populations (0.103) was much higher than that of 16 diploid populations (0.008) already investigated by Ohnishi and Asano (1999), and higher than that of other self-fertilizing plants (0.074) (see the review of Hamrick and Godt, 1989). The diploid Changbo-2 population had the highest average heterozygosity (0.131). This value was much higher than that reported in other self-fertilizing plants (0.074).

Despite the close distribution of the three diploid populations in the Batang district (Fig. 1), the diploid Changbo-2 population was found to have a long genetic distance from the two other populations. This indicates that the diploid Changbo-2 population was highly differentiated from the other two populations, and the morphologically distinct red-winged plants may hold the key to an understanding of why such a population has occurred.

Plants that are putatively considered to be hybrid plants were also observed in the diploid Changbo-2 population. These plants have a mosaic of characters found in the two types (i.e. normal type seeds with the vegetative parts of the red-winged type). The red-winged type and the normal type may have crossed by chance, and the resulting hybrid plants have increased in number. This would strongly suggest that the red-winged plants do not belong to a new species, even though the morphological differences observed and the genetic distance between the red-winged type and the normal type (0.647) suggests that

these two types should belong to different species.

A more detailed analysis, including crossing between the two types of plants, is required to verify that these red-winged plants arose as mutations in the population. We hope that future research will help clarify the nature of these unique plants.

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A preliminary report on inter-variety hybridization between Korean landraces and Canadian cultivars of buckwheat (*Fagopyrum esculentum* Moench)

Byoung Jae PARK[1], Soo Yong CHOI[2], Ju Kyoung LEE, Sun Hee WOO[3] and Cheol Ho PARK[1]

[1] *Department of Plant Biotechnology, Kangwon National University, Chunchon 200-701, Korea*
[2] *National Seed Management Office, Iksan 570-890, Korea*
[3] *Department of Agronomy, Chungbuk National University, Cheongju 361-763, Korea*

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ABSTRACT

The present study was an attempt to produce a promising buckwheat cultivar with higher yield and good quality by crossing Korean landraces to Canadian cultivars. Four Korean landraces (Bongpyoung, Cheongsong, Kunwe, and Youngwol) and three Canadian cultivars (Koban, Koto, and Mancan) were reciprocally crossed between pin and thrum plant types within each cross combination. Seeds of the Canadian cultivars were all larger and heavier than the Korean landraces. Differences in seed length/width ratio between the Korean landraces (average 1.74) and Canadian cultivars (average 1.45) indicated that seeds of the Canadian cultivars are rounder rather than the Korean landraces. Dry weight per plant was slightly higher in the Canadian cultivars (8.0 g) than in the Korean landraces (7.7 g). Most of hybrid lines expressed an intermediate seed size between the two parents. Two lines from controlled pollination within each line and three lines from open pollination among the lines had heavier 100 seed weight when compared to other breeding lines. An increase in seed weight in the hybrid lines appeared to be mostly attributed to the larger seeds of the Canadian cultivar, Koto.

INTRODUCTION

Buckwheat (*Fagopyrum esculentum* Moench) has been grown in Korea as a minor crop but has recently been attracting peoples' attention because of its bio-active value which has been newly elucidated (Choi et al., 2003; Kreft et al., 2003). In spite of the increased interest and utilization of buckwheat, most farmers in Korea do not grow buckwheat because it still has low productivity, hence leads to lower income when compared to other major crops. Buckwheat normally yields 90 to 107 kg/10 a, although yields of 150 kg/10 a have been produced under favorable and well-managed field conditions (Choi et al., 1998). The cultivated area and yield of buckwheat in Korea has been markedly decreasing in recent years. In 2002, the cultivated area and yield of buckwheat were estimated to be 3,466 ha and 3,725 M/T, respectively. This amount of production shows that the self-sufficient rate of buckwheat in Korea is less than 30%.

The improvement in culture practices to increase yields of buckwheat in Korea has some severe limitation as Korea usually has heavy rainfall during the summer growing period which results in lodged plants. Therefore, the development of a high-yielding variety is very imperative to improve the productivity of buckwheat and to meet the demand for domestically produced buckwheat. However, there previously has been limited accomplishment in buckwheat breeding in Korea as the genetic resources are limited. Most cultivars recommended for production in Korea were developed by selecting a segregating line from an original population from foreign cultivars which had been previously introduced into Korea. Many farmers in Korea still grow self produced landraces in which the seeds are small and the grain yield is very low. On the other hand, new cultivars of buckwheat with diverse characters have been released in many countries through the utilization of conventional or biotechnological breeding methods and from diverse genetic resources collected from around the world (Campbell, 2003).

Therefore, it was felt that we should develop new varieties with improved seed characters and yield to enhance the productivity of buckwheat in Korea through the introduction of genes for desirable genetic characteristics from superior varieties into local landraces. The final objective of this study is to produce a promising buckwheat cultivar with higher yield and good quality through crossing Korean landraces to Canadian cultivars.

MATERIALS AND METHODS

The initial effort in developing new cultivars was to make crosses between Korean landraces and Canadian cultivars. Four landraces (*jaerae*) that had been collected from the counties of Bongpyoung, Chongsong, Kunwe, and Youngwol in Korea were crossed with three Canadian buckwheat cultivars, Koto, Koban, and Mancan. The

crosses which, were conducted under greenhouse conditions, were reciprocally made to produce F_1 hybrids for all cross combinations. Emasculation of the female parent was performed 24 hours prior to crossing. The stigmas of the female parents were pollinated with pollen grains from selected flowers from the male parent. Twenty to fifty seeds from progeny in the F_2 generation from each breeding lines were sown individual in pots. At the initiation of flowering, a pot containing a pin plant was put together with pot containing a thrum plant of the same or a different breeding line in a net case. Pollination and seed set from each combination was accomplished by wind movement as well as by flies which were captured and placed in to the net cases. Each F_3 breeding line was grown in a net case and the largest seeds were harvested. Selected breeding lines were produced up to the F_5 stage through legitimate (pin-thrum) pollination within the line and were evaluated for morphological features as well as for seed characteristics.

A second trial was performed using open pollination among the F_1 breeding lines (sib family) which had been produced from crosses between the Korean landraces and the Canadian cultivars in the first year. Breeding lines with larger seeds were selected from among the progeny and advanced through the F_2 to F_5 generations.

One hundred seeds and ten plants from each breeding that had been finally selected were evaluated for major agronomic characteristics which included seed size, seed weight, seed shape, seed color, plant height, number of leaves, number of branches, and the plant dry weight.

RESULTS AND DISCUSSION

Crosses between the Korean landraces and the Canadian cultivars were made in a large number of combinations to generate genetic variation. The four Korean landraces (Bongpyoung, Cheongsong, Kunwe, and Youngwol) and three Canadian cultivars (Koban, Koto, and Mancan) were reciprocally crossed between pin and thrum plant types within each cross combination.

8 lines were selected based on seed size and 100 seed weight (Table 1) among the progenies from the 24 cross combinations which were developed. The lines were maintained by controlled pollinations (single cross, backcross or double cross) within or between different cross combinations. Another group of 10 lines were selected from the open-pollinated population of hybrid plants developed from each cross combination or controlled cross (Table 2).

The seed size of Youngwol was the smallest among the Korean landraces, whereas that of Kunwe was the largest. Seed length ranged from 6.2 mm to 7.2 mm and seed width ranged from 3.4 mm to 4.1 mm in the Korean landraces (Table 3). The ratio of seed length/seed width was 1.64 in Cheongsong, 1.85 in Youngwol, 1.72 in Bongpyoung and 1.76 in Kunwe. The 100 seed weight of the Korean landraces was 2.5 g (Youngwol), 2.7 g (Cheongsong), 2.9 g (Bongpyoung), and 3.2 g (Kunwe) respectively. Seeds of the Canadian cultivars were all larger and heavier than those of the Korean landraces. Seeds of Mancan, which were the largest, were 7.2 mm long and 5.2 mm wide on the average. Seeds of Koban were 6.4 mm in length, 4.0 mm in width, and 3.5 g in 100 seeds weight. However, the 100 seed weight of Mancan and Koto were 3.6 g and 3.8 g, respectively. The ratio of seed length/seed width of the Canadian cultivars were 1.38 (Mancan), 1.36 (Koto), and 1.60 (Koban) respectively. Differences in seed length/seed width ratio between the Korean landraces (1.74) and the Canadian cultivars (1.45) indicates that seeds of the Canadian culti-

Table 1. Buckwheat breeding lines selected from each cross combination between Korean buckwheat landraces and Canadian cultivars for which pollination was controlled within each combination.

Line No.	Type of cross	Cross combination
101	single	Bongpyoung×Koto
102	single	Bongpyoung×Koban
103	backcross	(Bongpyoung×Koban)×Koban
104	single	Cheongsong×Koto
105	single	Youngwol×Koto
106	double	(Chongsong×Koto) ×(Youngwol×Koban)
107	modified backcross	(Youngwol×Koto)×Koto ×(Youngwol×Koto)
108	double	(Cheongsong×Koto) ×(Youngwol×Koto)

Table 2. Buckwheat breeding lines selected from each cross combinations between Korean buckwheat landraces and Canadian cultivars for which pollination was open among the cross combinations.

Line No.	Type of cross	Cross combination
501	single	Bongpyoung×Koban
502	single	Bongpyoung×Koto
503	backcross	(Bongpyoung×Koban)×Koban
504	double	(Cheongsong×Koto)× (Youngwol×Mancan)
505	single	Cheongsong×Koto
506	double	(Chongsong×Koto)× (Youngwol×Koban)
507	single	Youngwol×Koto
508	double	(Youngwol×Mancan)× (Bongpyoung×Koban)
509	backcross	(Kunwe×Koto)×Koto
510	double	(Youngwol×Koto)× (bongpyoung×Koto)

Table 3. Buckwheat seed characteristics of Korean landraces and Canadian cultivars, and their F_5 hybrid lines

Cultivars or Hybrid lines	100 seed weight*	Seed length (mm)	Seed width (mm)
Cheongsong	2.7c	6.4±0.3c	3.9±0.2c
Youngwol	2.5c	6.3+0.3c	3.4±0.2c
Bongpyoung	2.9bc	6.2±0.2c	3.6+0.2c
Kunwe	3.2bc	7.2±0.1b	4.1±0.9c
Mancan	3.6b	7.2±0.8b	5.2+0.1b
Koto	3.8b	6.5±0.1bc	4.7±0.1c
Koban	3.5b	6.4+0.4c	4.0±0.1c
101	4.4a	7.4±0.5b	5.7±0.1a
102	3.8b	7.8+0.1a	4.6±0.2c
103	2.7c	7.2+0.2b	4.7±0.2c
104	3.4b	7.2±0.1b	5.1+0.2b
105	3.7b	6.5±0.1bc	4.4+0.1c
106	3.2bc	7.5±0.2a	4.6+0.2c
107	4.1ab	6.6±0.1bc	4.9±0.3b
108	3.7b	7.0±0.3b	5.2±0.2b
501	3.7b	6.8+0.2b	4.7±0.3c
502	4.0ab	6.7+0.2b	5.0±0.2b
503	3.4b	7.1+0.2b	5.4±0.2b
504	3.6b	7.1±0.5b	4.9+0.1b
505	3.7b	7.3±0.6b	4.8±0.2b
506	2.9bc	6.2+0.2c	5.0±0.1b
507	4.2ab	7.1±0.2b	5.1±0.2b
508	3.4b	6.4±0.2c	6.2±0.3a
509	3.7b	6.5±0.1bc	5.2+0.2b
510	4.0ab	6.2±0.2c	5.1±0.4b

*Duncan's multiple range test at the 5% level.

vars have a shape that is short-oval to semi-round when compared to the shape of the Korean landraces.

Morphological characteristics, such as plant height, number of leaves, number of branches, and fresh and dry weight of the plants, were compared between the Korean landraces and the Canadian cultivars used in the crosses (Table 4). The Canadian cultivars were generally taller than the Korean landraces. Dry weight per plant was slightly higher in the Canadian cultivars (8.0 g) than in the Korean landraces (7.7 g). This appeared to be due to broader leaves on the Canadian cultivars at an early growth stage.

The seed characteristics of the hybrid lines varied in seed size and 100 seed weight and remained almost the same from generation to generation. Most of the hybrid lines showed an intermediate seed size between that of the two parents. Two lines from the controlled pollination within each line and three lines from open pollination among the lines, had a heavier 100 seed weight when compared to the others (Fig. 1). The increase in seed

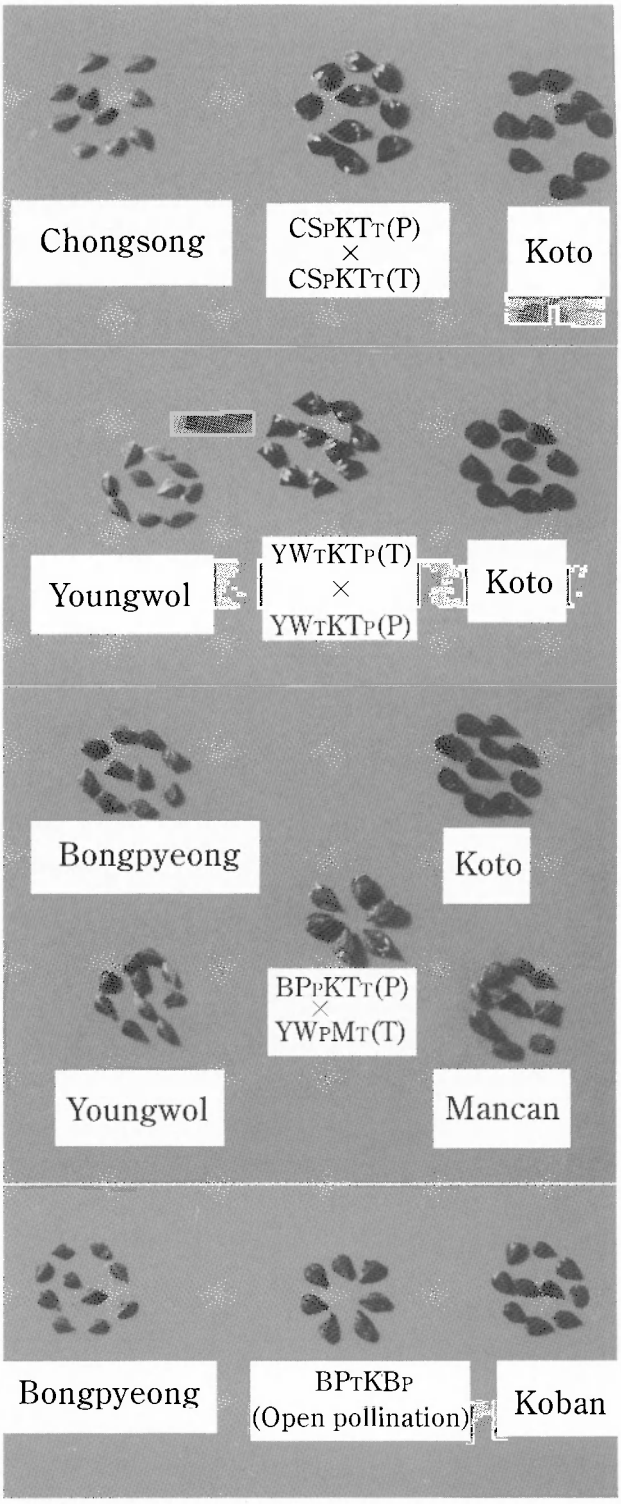

Fig. 1. Seed morphology of hybrid lines obtained from crosses between Korean landraces and Canadian buckwheat cultivars.

weight in the hybrid lines appeared to be mainly attributed to the large seeds of the Canadian cultivar, Koto. The seed length/width ratio of the hybrid seeds ranged from 1.30 to 1.70 (1.47 in average) in the progenies obtained by controlled pollination and from 1.03 to 1.52 (1.32 in average) in the progenies produced from open-pollination.

Table 4. Morphological characteristics of parental plants of Korean landraces and Canadian buckwheat cultivars evaluated in 1999

Parent	Plant length (cm)	No. of leaf (/plant)	No. of branch (/plant)	Freshweight (g/plant)	Dry weight (g/plant)*
Cheongsong	106.7±8.5b	38.7±17.5b	5.0+1.0a	18.0±3.0b	6.0+1.0bc
Youngwol	101.7+7.5c	33.3+10.6c	4.0+1.0bc	14.3+2.1c	5.7±1.5c
Bongpyoung	105.0+5.0b	56.3±6.0a	4.3±1.5b	18.3±2.9b	6.7±1.5b
Kunwe	109.0±13.1ab	37.3+5.1b	3.7±1.2c	17.3±2.1b	6.3±1.5b
Mancan	116.0±6.6a	38.7±8.6b	3.7±0.6c	15.3±3.2c	8.0±1.0a
Koto	115.3±6.5a	39.0±24.4b	3.3±0.6d	22.3±3.2a	8.0±2.0a
Koban	110.3±5.5a	23.7+2.5d	5.0±1.0a	12.7+18.8c	8.0+1.0a

*Duncan's multiple range test at the 5% level.

Table 5. Morphological characteristics of hybrid lines (F_5) between Korean landraces and Canadian buckwheat cultivars evaluated in 2002

Hybrids line	Plant length (cm)	No. of leaf (/plant)	No.of branch (/plant)	Fresh weight (g/plant)	Dry weight (g/plant)*
101	128.1±32.6a	80.0±40.0c	19.7±7.0b	159.0±73.1b	20.3±9.6b
102	121.0+12.0b	95.7±42.7b	19.7±7.0b	159.0±73.1b	26.3±14.0a
103	113.3±27.9c	97.0±73.7b	23.0±6.1ab	82.7±48.6c	18.0±2.0b
104	123.0±27.9ab	67.0±50.3c	14.0±6.9c	82.7±48.6c	12.0±2.0d
105	122.7±19.4ab	140.3±46.5b	28.3±8.1a	206.0+72.7a	31.7±2.5a
106	113.7±33.6c	87.0+78.9bc	20.7±9.0b	114.7±11.4b	17.7±2.5b
107	118.7+17.6b	55.0±12.3d	19.7±7.1b	63.3±20.6c	10.3±1.2d
108	117.0+23.1b	92.7±62.0b	22.0±9.5b	132.7±10.6b	18.0±2.0b
501	116.7+8.5b	66.0±12.5c	19.3±3.1b	52.0±3.0cd	15.0+5.0bc
502	129.3±33.6a	34.0±3.5e	13.7±5.0c	46.7±18.0d	11.0+1.0d
503	121.7±5.9b	48.7±12.1de	13.0±2.6c	65.3±18.8c	13.0±1.0cd
504	128.0±5.6a	193.7±86.0a	23.7+7.6ab	151.7±50.5b	26.3±0.2a
505	120.7±20.7b	84.7+27.3cbc	18.7±6.7b	57.7±4.2cd	12.3±2.5cd
506	125.0±12.2a	107.0±57.0b	18.3±3.5b	122.7±65.2b	21.0±12.8b
507	121.3+14.5b	79.3±7.0bc	20.0±10.4b	64.3±2.1c	14.3+3.5c
508	122.7+16.8ab	103.0±4.4b	21.0±1.0b	110.7±59.2b	20.0±5.6b
509	118.7+7.8b	27.3±15.9e	11.0±3.6c	42.7±7.5d	11.7+1.5d
510	123.7±17.6ab	64.0±13.5c	25.3+2.1a	124.7+61.8b	26.0±7.0a

*Duncan's multiple range test at the 5% level.

The morphological characteristics of the hybrid lines varied according to genotype and generation. Hybrid plants in the F_5 generation, from the controlled pollination and open-pollination populations, were investigated for morphological characteristics, such as plant height, number of leaves, number of branches, and fresh and dry weight of the plants (Table 5). In 2002, the hybrid plants grew vigorously and had increased plant height, number of leaves, number of branches, and fresh and dry weight per plant as compared to the parental plants which were used when the initial crosses were made in 1999.

From a practical point of view, comparison in growth and yield components between the parents and the hybrids was not possible in this study as the seeds of the parental plants were unfortunately not produced through legitimate pollination within same genotype until the fifth generation. Seed set of the hybrid plants was good inside the net cases as well in the outside open field. Alekseeva (1994) demonstrated that inter-variety hybrids between long stemed and high-branching Altiramosum and dwarf forms were the most productive and thus promising plants in terms of yield productivity and grain quality were selected from the hybrid materials. Our trial also used inter-variety hybridization which was aimed at the improvement of seed characteristics. We have selected 10 promising breeding lines from the results of this study

and further studies are in progress to develop new varieties to contribute to improved productivity in buckwheat in Korea.

Under field conditions, it is hard to estimate and compare the seed yield among the lines developed in this study as insect behavior is related to the final seed set following legitimate pollination. The yield which may be obtained in the farmer's field from each breeding line can only be determined after further studies. However, we could identify a few potential superior hybrid lines with desirable seed shape and larger seeds which may contribute to a higher yield of dehulled buckwheat groats for processed. Such new lines are expected to contribute to the improvement of Korean buckwheat landraces through the introduction of desirable genes from the Canadian buckwheat cultivars.

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Fagopyrum 21: 21–25 (2004)

Centromeric location of the *S*-locus in buckwheat

Jotaro AII*[1], Mio NAGANO[2], Shinji KAWASAKI[1] and Taiji ADACHI[3]

[1] *National Institute of Agrobiological Sciences (NIAS), 2-1-2, Kannon-dai, Tsukuba, Ibaraki, 305-8602, Japan*
[2] *Department of Biology, Kochi University, 2-5-1 Akebono-cho, Kochi, 780-8520, Japan*
[3] *Laboratory of Plant Genes and Physiology, Graduate School of Agriculture and Biological Sciences, Osaka Prefecture University, 1-1 Gakuen-cho, Sakai, Osaka, 599-8531, Japan*

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ABSTRACT

We recently developed a high-resolution genetic linkage map and constructed a BAC based physical map with intermittent contigs around the *S*-locus in buckwheat. In order to determine the physical location of the *S*-locus, sequence analysis and fluorescence *in situ* hybridization (FISH) using a BAC clone (145E12) probe, which was closely linked to the *S*-locus, was performed on common buckwheat, *Fagopyrum esculentum*, chromosomes. DNA sequences of the *S*-linked BAC clones and molecular markers revealed several repetitive sequences, some of which showed high homology to the centromeric region of *Arabidopsis* and rice. The *S*-locus linked BAC clone, 145E12, detected signals close to the expected centromere of the chromosome. These data suggest that the *S*-locus of buckwheat may be located very close to the centromeric region which could be one of the causes of suppressed recombination within the *S*-super gene complex, the *S*-locus in buckwheat.

INTRODUCTION

Self-incompatibility is a cell to cell recognition system that prevents inbreeding in flowering plants to maintain a wide variability in the genome against various stresses. As Darwin (1877) had already noticed, some plants have independently evolved an elegant system promoting insect mediated outcrossing, which may be combined with heterostyly. In buckwheat, a mechanism of self-incompatibility which has a dimorphic sporophytic system is thought to be a novel regulated one due to its self-pollen rejection behavior. The major cultivated species, *Fagopyrum esculentum*, is self-incompatible with heterostylic morphology, however, the wild species, *F. homotropicum*, is self-compatible and homostylic. Genetic analysis of the cross between *F. esculentum* and *F. homotropicum* indicated that the homostyly and self-compatibility of *F. homotropicum* are controlled by a single *S*-super gene complex, the *S*-locus (Woo et al., 1999). Buckwheat can be utilized as a good model plant to analyze the mechanism of heterostyly and self-incompatibility, since buckwheat's life cycle is short, 2–3 months, and a simple efficient transformation system has been established (Kojima et al., 2000).

We have recently developed a saturated molecular linkage map and a BAC based physical map with intermittent contigs around the *S*-locus (Aii et al., in preparation). However, the cytological characteristic of the *S*-locus is still unknown. The purpose of the present study was to determine the location of the *S*-locus on the chromosome and to characterize the regions close to the locus. We first sequenced several BAC clones and molecular markers closely linked to the *S*-locus. Then we performed FISH on the chromosomes of common buckwheat *F. esculentum* using the BAC clone (145E12), which is closely linked to the *S*-locus. In this paper, we describe the location of the *S*-locus in buckwheat and discuss the location of the *S*-super gene complex.

MATERIALS AND METHODS

Plant material, genomic DNA isolation and Southern blot analysis

Common buckwheat, *F. esculentum*, variety Miyazaki zairai and its closely related wild annual species, *F. homotropicum*, were grown in a greenhouse at 25°C. *F. esculentum* has a dimorphic sporophytic self-incompatibility system, whereas *F. homotropicum* is a homomorphic self-compatible species. Genomic DNA isolation and southern blot analysis were performed using the methods described by Nagano et al. (2001). Probe labeling, hybridization and DNA detection were carried out using AlkPhos Direct (Amersham) following the manufacturer's instructions.

Molecular markers and BAC clone sequence

Molecular markers and BAC clones, which had previously been mapped close to the *S*-locus (see Fig. 1), were sequenced using a Big Dye Terminator ver. 3 (Applied Biosystems) and an automated DNA sequencer ABI

* Corresponding author

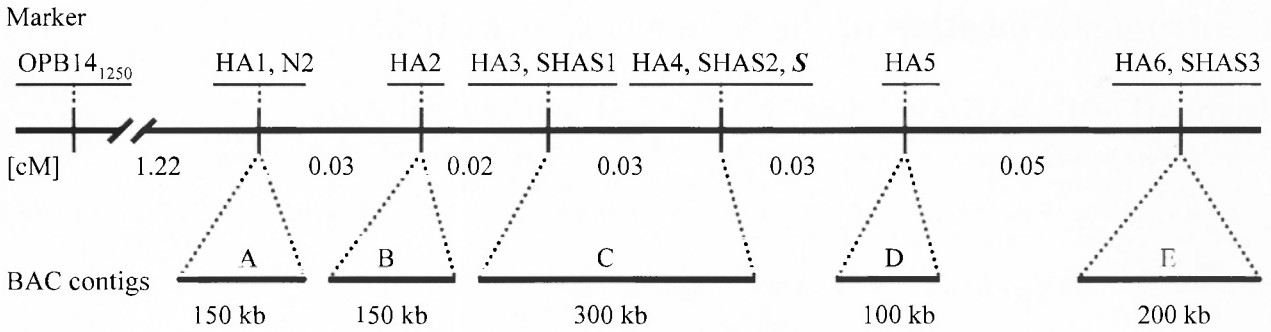

Fig. 1. Schematic representation of the molecular genetic linkage map around the *S*-locus in buckwheat and the BAC based physical intermittent contigs. The genetic linkage map was constructed from 3,112 F_2 plants from an inter-specific hybrid cross between *F. esculentum* and *F. homotropicum*. The molecular marker, OPB14$_{1250}$ was from RAPD (Aii et al.,1998) and the other markers are from Anchor-SAMPL (SHAS1, SHAS2 and SHAS3) and AFLP (HA1, HA2, HA3, HA4, HA5 and N2) (Aii et al., in preparation). The map distance is as indicated by Kosambi cM. The physical map was constructed using a BAC library developed from *F. homotropicum*. Each BAC contig (A–E) consisted of at least three independent BAC clones and each clone overlap was verified by sequencing or PCR analysis with the approximate size being listed under that portion of the map. The BAC clones, 145E12 and 193F11, which have highly homologous sequences to centromeric specific sequences in other plants, are in the contig A and B, respectively.

PRISM 3100 (Applied Biosystems). The program BLASTN homology search software was used for comparison of the molecular markers and the BAC clone sequences with other plant sequences in the GenBank database.

Chromosome preparation for FISH

F. esculentum seeds were germinated on a sheet of moistened filter paper in 9 cm petri dishes kept at 25°C in the dark. Root tips emerged from the germinating seeds after 2 to 5 days. Approximately 1 cm of the root tip was cut off and placed into 8-hydroxyquinoline (2.5 mM) for 4 hr under normal room light conditions at 25°C. They were then fixed in methanol/acetic acid 3:1 (v/v) at 4°C for at least 3 days. The root tips were then cut into 0.5 mm pieces and digested with an enzyme cocktail (0.1% cellulase, 0.05% pectolyase Y-23, 0.05% macerozyme: pH 5.7) at 37°C for 1 hr. They were then placed on prepared glass slides, after the removal of the enzyme cocktail, and 1 drop of 45% CH_3COOH was applied after which they were kept for 10 min at room temperature. The root tips were then gently squashed by finger pressure on the cover glass after heating to hydrolysis. The cover glasses were removed and the slides were then kept at –80°C for 1 hr and then aged at 25°C for 1–7 days before being evaluated.

FISH analysis

In situ hybridization was performed following the method described previously (Suzuki et al., 2001) with slight modifications. The probe DNA, the BAC clone DNA 145E12, was extracted by an alkali-SDS method and purified by cesium gradient centrifugation. The rDNA clone was extracted utilizing the alkali-SDS method. The extracted clone was labeled with Dig-11-dUTP, using a Dig-Nick Translation Mix kit (Roche Diagnostics) and a Biotin-Nick Translation Mix kit (Roche Diagnostics). Chromosomal DNA, fixed on the glass slide, was denatured in 70% formamide-2×SSC for 2.5 min at 70°C and dehydrated in an ethanol series (70%, 80%, 100%) at –20°C for 5 minutes at each level. The hybridization mixture (50% formamide, 10% dextran sulfate, 50 μg of blocking DNA, 2×SSC, 1 μg of the labeled BAC DNA) was denatured for 10 minutes at 100°C and quenched immediately in ice for at least 10 minutes. 20 μl of the mixture was applied to the slides. Hybridization was allowed to proceed overnight in a moist chamber at 37°C. After hybridization, the slides were washed with 2×SSC at room temperature for 5 min, 50% formamide-2×SSC at 37°C for 15 min, 2×SSC at room temperature for 15 min, 2×SSC at room temperature for 15 min, 1×SSC at room temperature for 15 min, and 4×SSC at room temperature for 5 min. Digoxigenin was detected with rhodamine-conjugated sheep anti-Dig Fab fragments. The slides were incubated with 10 μg/ml rhodamine-conjugated anti-Dig in a detection buffer containing 4×SSC for 10 min, 4×SSC for 10 min, and 2×SSC for 5 min, all at room temperature. The slides were then mounted in a fluorescence antifade solution. DAPI (4' 6-diamidino-2-phenylindole) was used for chromosome DNA counterstaining by adding it to the antifade solution at 20 mg/ml. Digital imaging analysis was carried out using Lumina Vision.

RESULTS AND DISCUSSION

We have recently developed a saturated molecular linkage map and a BAC based physical map with intermittent contigs around the *S*-locus (Aii et al., in preparation). The scheme as depicted in Fig. 1 represents the molecular

markers and BAC contigs positions around the *S*-locus. We then carried out DNA blot analysis, using molecular markers linked to the *S*-locus as the probes, in order to characterize its origin. The majority of the molecular markers which were found to be around the *S*-locus demonstrated a multi-copied origin and only a few markers were found to be of low-copied origin (Fig. 2). The sequences of the molecular markers and the BAC clone ends, which were components of the BAC contigs from A to E, showed that many of them had repetitive sequences similar to copia or gypsy type retrotransposons. In particular, the marker $OPB14_{1250}$, the BAC clone end sequences of 145E12, 193F11 and 112C6, from contig A, B and E, respectively, were found to be highly homologous to those in the centromeric region of other model plants (Table 1).

In order to detect the physical location of the *S*-locus on the chromosomes of buckwheat, we performed FISH using a biotin-labeled BAC clone (145E12), which consisted of contig A, as a probe. The BAC clone (145E12) was detected as red fluorescent signals on the metaphase chromosomes (2n=16), which was composed from the image of the DAPI stained chromosomes (Fig. 3). As the BAC clone contained many repetitive sequences, it was difficult to detect a bright signal on the chromosome. After the experiments had been repeated several times and similar results were obtained with all having the same doublet signals at the identical position, we concluded that this must be the location of the *S*-locus. Based on the chromosome length, the position of the FISH signal was determined to be very close to the centromeric region of the chromosome in buckwheat.

Recent mapping results have suggested that the chromosomal location of the *S*-locus may be a major factor in the suppression of recombination in this region. The *S*-locus in the family Solanaceae has been shown to be a *S*-locus containing several genes, which controlled not only the self-incompatibility response but also the developmental processes of reproductive organs, and was located close to the centromere (Bernacchi and Tanksley, 1997; Entani et al., 1999). This is suggestive of heteromorphic self-incompatibility systems in which multiple *S*-locus linked genes controlling morphological traits and genetic identity act together to result in the self-incompatibility responses (de Nettancourt, 1977). In the present study, we have characterized molecular markers and BAC clone end sequences around the *S*-locus in buckwheat. The majority of the molecular markers and BAC clone end sequences revealed heavily repetitive sequences and some of them showed high homology to centromeric specific sequences in other model plants. Furthermore, the FISH analysis, using the BAC clone (145E12), which was closely linked to the *S*-locus as the probe, demonstrated that the hybrid signal occurs close to the expected centromere of the chromosome. These

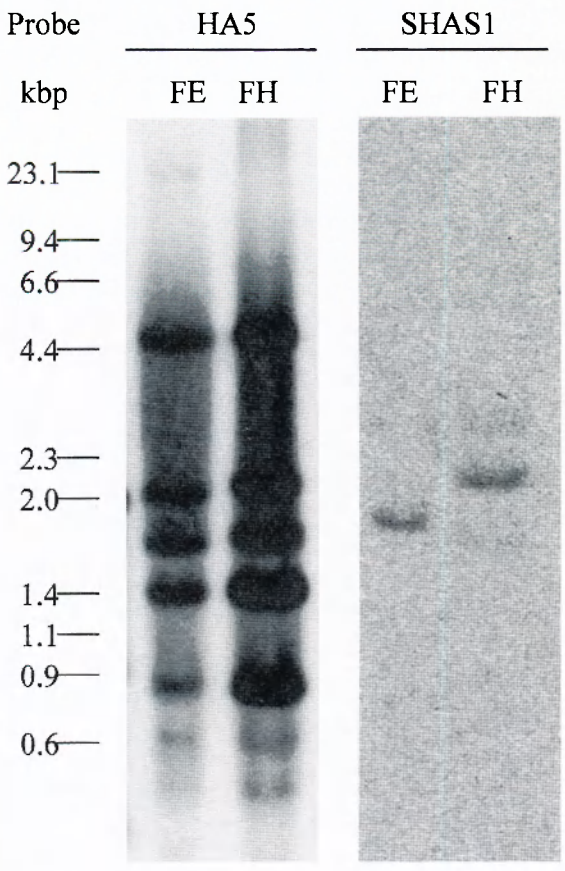

Fig. 2. Characterization of molecular markers around the *S*-locus by genomic DNA gel blot analysis. The genomic DNAs from *F. esculentum* (FE) and *F. homotropicum* (FH) were digested with *Eco*RI. The probes used for hybridization are indicated at the top of each profile. The molecular mass markers, in kilobases, are indicated on left.

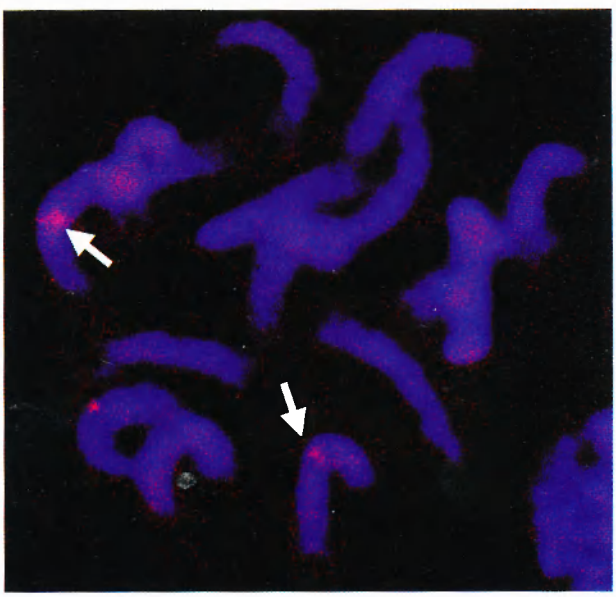

Fig. 3. FISH analysis of *F. esculentum* using the BAC clone, 145E12, as a probe. The hybrid signals are as indicated by the arrows.

Table 1. Comparison (BLASTN) of the molecular marker, $OPB14_{1250}$, and BAC clone end sequences in buckwheat with other known plant centromeric sequences

Query names	Similarity to/accession	Position on the original accession	Position on query	Identity [%]
$OPB14_{1250}$	*O. sativa*, centromeric satellite and non-functional centromeric specific retrotransposon/AY101510	3556-3621	1037-1102	89
	Z. mays, CRM centromeric retrotransposon/AY129008	4221-4283	1040-1102	84
	A. thaliana, chromosome 4 centromere region/AB073162	1957-2006	893-942	86
		1836-1783	1061-1114	85
145E12M13F[1]	*A. thaliana*, chromosome 1 centromere region/AB062093	67006-67091	1780-1865	84
		67180-67202	1954-1976	100
	A. thaliana, chromosome 1 centromere region/AB062087	43556-43627	1475-1546	84
		67791-67828	2050-2087	89
	A. thaliana, chromosome 4 centromere region/AB073156	55202-55262	1780-1865	83
	A. thaliana, chromosome 5 centromere region/AB046431	77823-77872	1495-1544	88
		41438-41529	1648-1739	81
		77502-77662	1705-1865	79
		77280-77317	2050-2087	92
		42322-42365	2548-2591	88
193F11M13F[2]	*A. thaliana*, chromosome 1 centromere region/AB062093	66776-66810	728-762	91
	A. thaliana, chromosome 4 centromere region/AB073156	23559-23665	655-762	84
	A. thaliana, chromosome 4 centromere region/AB073163	7698-7724	827-853	96
	A. thaliana, chromosome 5 centromere region/AB046433	67713-67739	827-853	96
112C6M13Rv[3]	*A. thaliana*, chromosome 5 centromere region/AB046439.1	72249-72216	235-268	91

[1] The BAC clone, 145E12, is in contig A. [2] The BAC clone, 193F11, is in contig B. [3] The BAC clone, 112C6, is in contig E.

results indicate that the *S*-locus in buckwheat must be located near the centromeric region. In another recent study, we developed a marker clustering map around the *S*-locus in high-density molecular linkage (Aii et al., 1998). Yasui et al. (2004) has also reported observing several markers clustered around the *S*-locus in their AFLP linkage map of the buckwheat whole genome and concluded that its location was near the center of linkage group 1. These reports support the centromeric location of the *S*-locus in buckwheat as AFLP marker clustering has been observed at the centromeric region of the chromosome in other plant species (Qi et al., 1998; Castiglioni et al., 1999). In *Primula*, which is a distylous self-incompatibility species, the *S*-locus was found to be located near the centromere and interfered with recombination in this region (Dowrick, 1956). Thus, we could conclude that the *S*-locus of buckwheat is located close to the centromere in buckwheat, in a similar manner as in Solanaceae or *Primula*, and this could well be the major cause of the suppressed recombination among the complex components.

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Isolation of a VIVIPAROUS 1/ABSCISIC ACID-INSENSITIVE 3 homologue from common buckwheat

Tomoko TOYAMA[1]*, Shingo NAKAMURA[2] and Makoto YAMAMORI

National Agriculture Research Center for Tohoku Region (NARCT), Morioka 020-0198, Japan
[1] Present address: Department of Medicine, Osaka University, Osaka 565-0871, Japan
[2] Present address: National Institute of Crop Science, Tsukuba 305-8518, Japan

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ABSTRACT

A *VP* (*Viviparous*) *1/ABI* (*Abscisic Acid-Insensitive*) 3 gene encodes a pivotal transcription factor regulating the expression of abscisic acid (ABA)-responsive genes and controls seed development and germination in plants. It has been shown that mutations in the *VP1/ABI3* gene causes vivipary, germination on the mother plants before harvest. The full length-sequence of the VP1/ABI3 homologue was determined using thermal asymmetric interlaced PCR (TAIL-PCR) in common buckwheat (*Fagopyrum esculentum* Moench cv. Hashikamiwase). The buckwheat *VP1/ABI3* (*BwVP1*) gene was deduced to encode for a protein with a predicted molecular mass of 84.9 kDa, and which displayed high homology to VP1/ABI3 homologues of other plants in four conserved domains. It has been shown that a single *VP1/ABI3* gene and other related genes are present in the buckwheat genome.

INTRODUCTION

Germination is one of the most important events as the beginning of the life cycle in plants, and this physiological phenomenon must be strictly controlled and performed in order for the plant to thrive under severe circumstances. Before germination, seeds experience a three step procedure of maturation, desiccation and dormancy. The seeds are not usually disconnected from the mother plant before completion of these steps. Consequently, the seedlings can grow properly after they are supplied with an adequate temperature and water supply. Premature sprouting while attached to the mother plant, 'vivipary', often results in desiccation and decay of the seedlings. Plants ordinarily possess complex and sophisticated mechanisms to prevent such a wasteful phenomenon (Holdsworth et al., 1999). However, vivipary is a serious problem among crops, particularly in some cereals, e.g. rice and wheat, due to the reduction in the quality of product produced due to the degradation of various seed storage compounds. Intensive studies have been performed on the immature germination of rice and wheat (Anderson et al., 1993; Bailey et al., 1999), however, there have been only a very small number of experiments that have evaluated germination and vivipary in buckwheat. Cormack (1952) found that the removal of the pericarp and seed coat resulted in the germination of dormant seeds of Tartary buckwheat. Vanden Born and Corns (1958) reported that gibberellic acid (GA) improved partially the germination of after-ripening seeds, but not of fully dormant seeds.

It has been shown that vivipary is caused by the lack of some factors that are necessary for regulatory mechanisms (Gale and Lenton, 1987). Many factors, such as plant hormones and proteins, including enzymes and regulatory factors, have been demonstrated to play various roles in controlling seed development and germination. It is well known that abscisic acid (ABA) is a potent inhibitor of germination in many plant species, whereas GA promotes germination (Hilhorst and Karssen, 1992). Several viviparous mutants of maize and *Arabidopsis* contain lower levels of ABA in their seeds, and these loci encode for enzymes involved in ABA biosynthesis. On the other hand, some viviparous mutants have normal levels of ABA and can germinate even in the presence of inhibitory concentrations of ABA, indicating a distinct function of these loci in the regulation of ABA-responsive events in seed germination (Hoecker et al., 1995; Parcy et al., 1997). The respective genes for these mutants have been shown to encode for a variety of proteins (reviewed by Finkelstein et al., 2002). Thus, elucidation of the regulatory mechanisms controlling seed maturation and germination is crucial for the study of vivipary.

The *Viviparous-1* (*VP1*) gene in maize and the *Abscisic acid-insensitive-3* (*ABI3*) gene in *Arabidopsis thaliana* are of particular interest. They are orthologous members belonging to a large group of transcription factors expressed specifically in developing seeds, Therefore, their homologues in plants have been called *'VP1/ABI3'* (McCarty et al., 1991; Giraudat et al., 1992; Finkelstein et al., 2002). The *VP1/ABI3* genes in monocots and dicots

* Corresponding author. FAX: +81-19-643-3655, E-mail: ttoyama@fbs.osaka-u.ac.jp

encode for a protein with a high degree of sequence similarity to each other, especially in four conserved domains (A1 and B1–B3, 37–98% at amino acid level). It is not only an intriguing protein that has been suggested to have direct involvement in ABA signaling, but has a most profound effect on seed development and germination, including the accumulation of storage proteins and lipids in seeds and the acquirement of desiccation tolerance (Nambara et al., 1995; Hoecker et al., 1995). The expression of the *VP1/ABI3* gene is strongly correlated with the level of seed dormancy (Jones et al., 1997; Nakamura and Toyama, 2001), indicating that VP1/ABI3 protein has a pivotal role in the arrest of germination in seeds (McCarty, 1995). Therefore, the isolation and characterization of the *VP1/ABI3* gene is an important step in elucidating the mechanism of vivipary. In the present study, we isolated a VP1/ABI3 homologue from common buckwheat using degenerate primers and thermal asymmetric interlaced PCR (TAIL-PCR). The isolated gene shares all the characteristics that are necessary to work as a member of the VP1/ABI3 homologues.

MATERIALS AND METHODS

Isolation of genomic DNA

A commercially cultivated Japanese variety of common buckwheat, var. Hashikamiwase, was routinely cultivated in soil in a greenhouse. Genomic DNA was isolated from leaves that were frozen in liquid nitrogen according to the procedure described by Maniatis et al. (1982). The concentration and purity of the DNA preparation were determined spectrophotometrically.

PCR amplification with degenerate primers

Three degenerate primers were synthesized based on the highly conserved amino acid regions of the VP1/ABI3 homologues (Hokkaido System Science, Japan). A PCR product was amplified by PCR with 20 mer-primers corresponding to the amino acid sequences RFWPNNK (3F: 5'-GNTT(C/T)TGGCCNAA(C/T)AA(C/T)AA-3') and QEGDFIV (5-2R: 5'-AC(A/G/T)AT(A/G)AA(A/G)TCNCC (C/T)TC(C/T)TG-3') in the B3 domain of the VP1/ABI3 proteins. A 2.4 kbp-PCR product was also amplified with primers corresponding to the amino acid sequences PDFPCMS (BW-F: 5'-CCNGA(C/T)TT(C/T)CC(A/T/C) TGCATGTC-3') in the A1 domain and 5-2R.

PCR was carried out using EX-Taq DNA polymerase (Takara Shuzo, Japan) with 0.2 mM each of dNTPs, 1×PCR reaction buffer, 2 mM primers and 1 mg gDNA in a final volume of 20 ml according to the manufacturer's protocol. The solution was reacted in a GeneAmp PCR System 9700 (Applied Biosystems, CA, USA) following the program: denaturing at 94°C, 3 min, 30 cycles consisting of the three consecutive incubations at 94°C for 1 min, at 55°C for 1 min, and at 72°C for 3.5 min. The PCR products were cloned into a Bluescript vector (Stratagene, CA, USA) after gel purification of the specific bands. The cloned DNA fragment was sequenced using ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems).

TAIL (Thermal asymmetric interlaced)-PCR

The TAIL method was carried out in accordance with the procedure of Liu and Whittier (1995). Three nested, target-specific primers were synthesized to obtain the 5'-end region. The sequences of the target-specific primers were: VP5'-1, 5'-TCATGCTGCTGCTCCCGACC-3'; VP5'-2, 5'-GAGCACCGCCCACGAGGCAG-3'; VP5'-3, 5'-CGAAGACGACATGCATGGAA-3' for the outer, middle and inner nested primers, respectively (Fig. 1). Primary TAIL-PCR reactions (20 μl) contained 1×PCR buffer, 0.25 mM each of dNTPs, 1 μg of gDNA, 1 unit of Ex Taq, 0.2 mM VP5'-1 and 4 mM one of three arbitrary degenerate (AD) primers (AD1; 5'-NTCGA(G/C)T(A/T) T(G/C)G(A/T)GTT-3', AD2; 5'-NGTCGA(G/C)(A/T)GA NA(A/T)GAA-3', AD3; 5'-(A/T)GTGNAG(A/T)ANCAN AGA-3'). PCR was carried out with the following program: denaturing at 95°C, 10 min, 5 cycles consisting of the three consecutive incubations at 94°C for 1 min, at 65°C for 1 min, and at 72°C for 2 min, 1 cycle at 94°C for 1 min, at 25°C for 3 min, and at 72°C for 2 min, and 15 nine-thermal-segment supercycles consisting of 2 cycles at 94°C for 30 sec, at 65°C for 1 min, and at 72°C for 2 min followed by 1 cycle at 94°C for 30 sec, at 45°C for 1 min, and at 72°C for 2 min.

Aliquots (1 μl) from 50-fold dilutions of the primary PCR products were applied directly to secondary TAIL-PCR reactions (20 μl) containing 0.2 mM VP5'-2 primer and 2 mM of the same AD primer used in the primary reaction. The PCR condition was denaturing at 95°C, 10 min and 13 cycles nine-thermal-segment supercycles consisting of 2 cycles at 94°C for 30 sec, at 64°C for 1 min, and at 72°C for 2 min followed by 1 cycle at 94°C for 30 sec, at 45°C for 1 min, and at 72°C for 2 min.

After amplification, aliquots (1 μl) from 10-fold dilutions of the secondary PCR products were applied directly to tertiary PCR including 0.2 mM VP5'-3 and 2 mM of the same AD primer. The PCR program was: denaturing at 95°C for 10 min, 20 cycles consisting of the incubations at 94°C for 30 sec, at 45°C for 1 min, and at 72°C for 2 min followed by extension at 72°C for 10 min. Amplification products from the reactions with 5'-2 and 5'-3 were analyzed using agarose gel electrophoresis.

The same TAIL-PCR protocol was also used to get the 3'-end region of the gene. The sequences of the target-specific primers were: VP3'-1, 5'-GTGGAGCTCCAG GTTCTGGC-3'; VP3'-2, 5'-TGGAGAACACTGGTGCG TAC-3'; VP3'-3, 5'-GGACTGCAGGAGGGCGACTT-3'

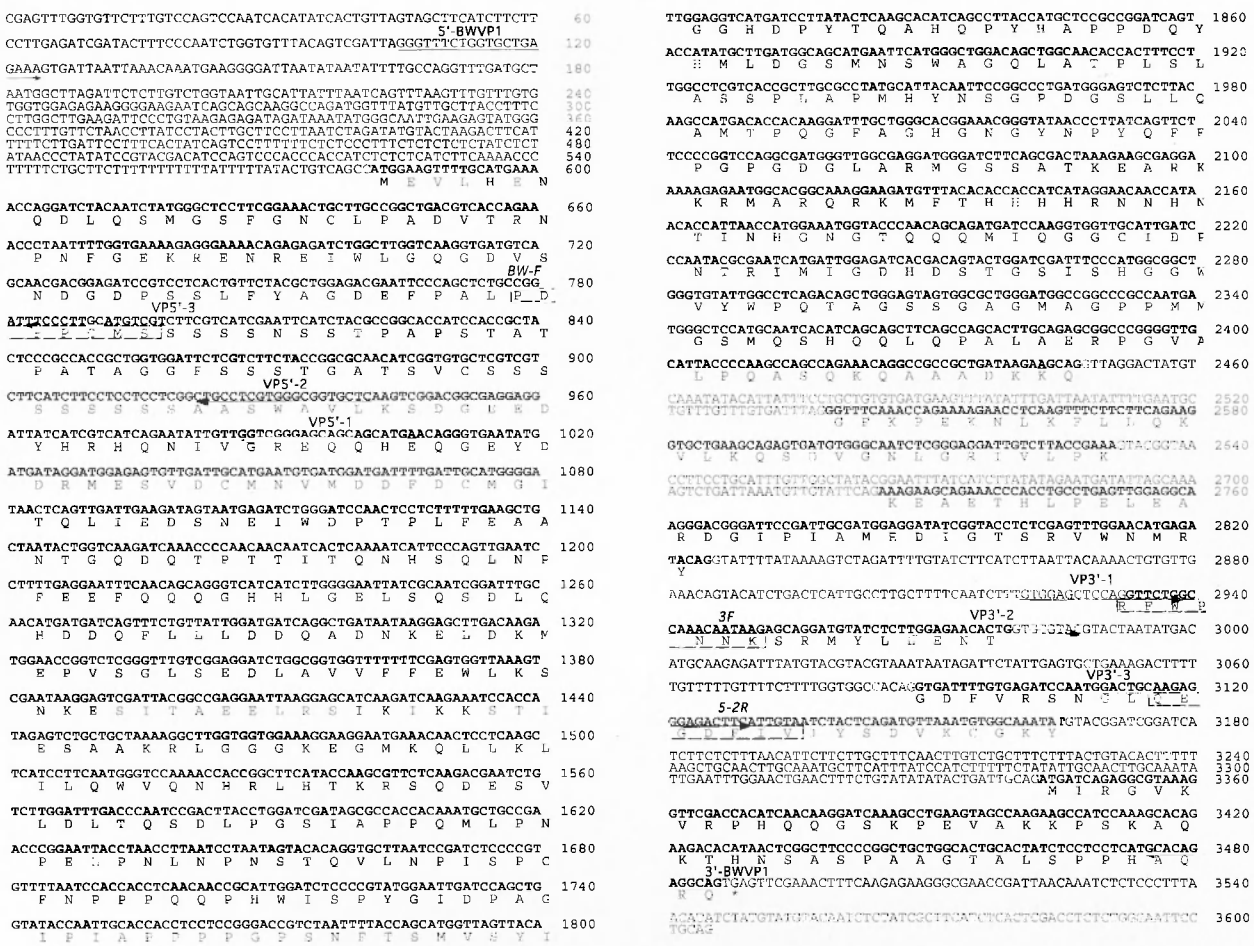

Fig. 1. Structure of the *BwVp1* gene (accession no. AB099513). The nucleotide sequence and putative amino acid sequence compiled from the PCR products with 5'- and 3'-BWVP1 primers and TAIL-PCR products are shown. The underlined nucleotide sequences indicate the positions of PCR primers. The amino acid sequences used for designing degenerate primers are boxed. The putative protein-coding nucleotide sequences are represented as bold letters.

(Fig. 1). The PCR products which were obtained were cloned into a pBluescript vector and sequenced.

To confirm the consecutive sequence derived from the TAIL-PCR products, another PCR reaction was performed with primers designed from the sequence of the 5'-TAIL PCR product (5'-BWVP1: 5'-GGGTTTCTGGT GCTGAGAAA-3') and the 3'-TAIL PCR product (3'-BWVP1: 5'-CGAACTCACTGCCTCTGTGC-3').

Southern blotting

Genomic DNA (10 μg) was digested with the restriction enzymes, *Bam*HI, *Eco*RI and *Xba*I (TAKARA, Japan) overnight at 37°C. The digests were electrophoresed in a 1.0% agarose gel. The DNA in the gel was denatured by a 30 min incubation in 0.25 M HCl, and subsequently neutralized by a 30 min incubation in a neutralization solution consisting of 0.5 M NaCl and 1.5 M NaOH. The DNA in the gel was transferred in 0.4 M NaOH and UV-cross linked onto a nylon membrane (Hybond™-N⁺, Amersham Pharmacia Biotech, UK). The membrane was prehybridized and hybridized with a fluorescent-labeled PCR product derived by 3F and 5-2R primers corresponding to the B3 domain of BwVP1 protein, using Gene Images random prime labeling and detection system (Amersham Pharmacia Biotech). The reaction was carried out overnight in a Hybridization buffer containing 5×SSC (20×SSC=0.3 M Na_3 citrate and 3 M NaCl), 0.1% SDS, 5% dextran sulfate and 5% Blocking reagent at 60°C according to the manufacturer's instructions. The blot was then transferred to the primary wash buffer (1×SSC and 0.1% SDS) and washed at 60°C with gentle agitation. Then the membrane was washed in a secondary wash buffer (0.5×SSC and 0.1% SDS) at 60°C with gentle agitation. The signal generation and detection was carried out with CDP-*Star* (Amersham Pharmacia Biotech) according to the protocols provided by the manufacturer. The membrane was rinsed with Buffer A {100 mM Tris-HCl (pH 9.5) and 300 mM NaCl} and incubated with a 10% Blocking reagent diluted with Buffer A at room temperature for 1 h with gentle agitation. After it was rinsed with Buffer A, the blot was incubated with 1/5000 anti-fluorescein alkaline phosphatase conjugate diluted with Buffer A at room temperature for 1 h with gentle agitation. The CDP-*Star*

detection reagent was dripped onto the membrane after it was rinsed three times with Buffer A including 0.3% Tween-20 and once with Buffer A. The membrane was exposed to an X-ray film to detect any signals.

RESULTS

There are four conserved domains (the acidic A1 region, and the basic B1–B3 regions) within the VP1/ABI3 proteins when they are compared among monocots and dicots. The degenerate primers, 3F and 5-2R, based on the amino acid sequences of the B3 region were used to amplify a PCR product of approximately 220 bp from the buckwheat genomic DNA. The nucleotide sequence of the PCR product showed a very high homology to the B3 domain in the putative exon regions (Fig. 2). The intron 5' and 3' splice site consensus sequences, GT and AG, were completely conserved at both ends of the putative intron (Brown, 1996; Simpson and Filipowicz, 1996). Another degenerate primer set, BW-F, designed from the A1 region and 5-2R, also amplified a PCR product of approximately 2400 bp. The PCR product also showed high homology to each conserved region (e.g. 60%–69% identity at the nucleotide level in the B3 region). We have designated this sequence in buckwheat as the *VP1/ABI3* homologous gene (*BwVP1*).

However, these nucleotide sequences lacked the 5'- and 3'-ends of the gene. 5'- and 3'-TAIL PCR methods were then applied to obtain the complete sequence of *BwVP1*. TAIL-PCR utilizes three nested specific primers in successive reactions together with shorter arbitrary degenerate (AD) primers (Liu and Whittier, 1995). In the process of TAIL-PCR, the primary TAIL-PCR with the first primer produces multiple unclear bands and the desired specific products are usually invisible. Such non-specific bands disappear in the subsequent secondary TAIL-PCR that the next primer replaces as the nested primer. The specificity of the PCR products is verified simply by the size shift of the secondary and tertiary PCR products in electrophoresis; the target products in the tertiary reactions must be smaller than those in the secondary reactions because of the utilization of nested primers (Liu et al., 1995).

We carried out 5'-TAIL PCR with VP5'-1, -2 and -3 and three AD primers (Fig. 1). The reaction with the AD1 primer gave a distinct 900-bp band in the secondary PCR, and the product of the tertiary PCR was smaller and consistent with the theory of TAIL-PCR (data not shown). The nucleotide sequence of the tertiary PCR product was determined and confirmed to contain the 5'-end of the *BwVP1* gene.

We also used TAIL-PCR reactions to determine the 3'-portion of the *BwVP1* gene. VP3'-1, -2 and -3 primers were synthesized and used in the PCR reactions as described above (Fig. 1). The reaction with the AD2 and VP3'-3 primers gave a distinct 500-bp product. This fragment corresponded to the 3'-end of the *BwVP1* gene.

The sequences of these PCR products compiled for the

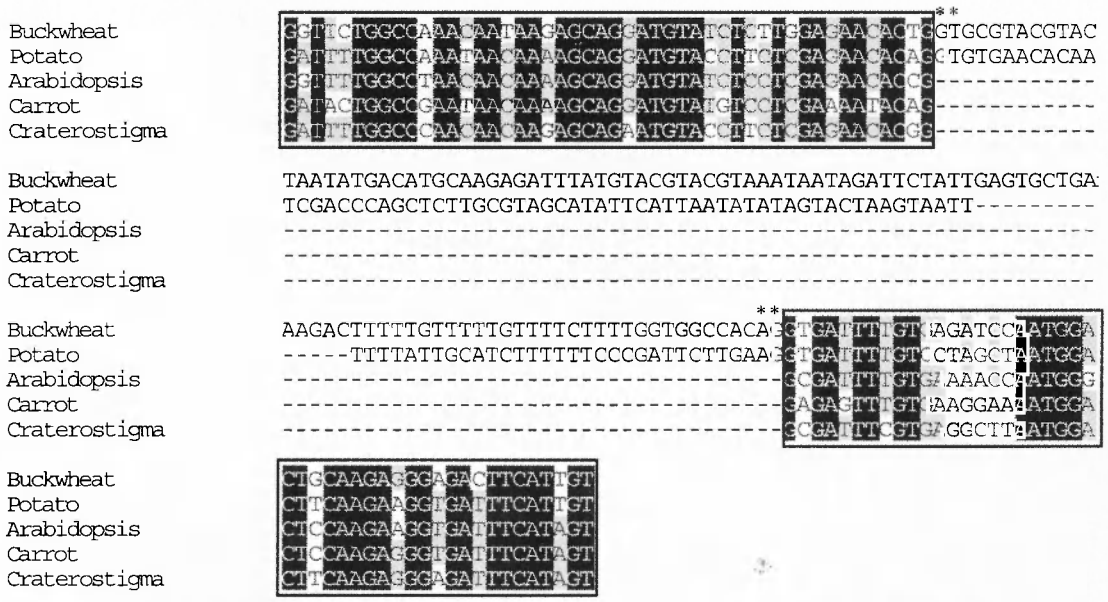

Fig. 2. Sequence similarity of the PCR product derived by the primers 3F and 5-2R to *VP1/ABI3* genes from dicots. *VP1/ABI3* genes [potato (AJ309218), *Arabidopsis thaliana* (X68141), carrot (AB005558), *Craterostigma plantagineum* (AJ000552)] were aligned with the computer program GENETYX-MAC (ver. 11.0). Note that potato *VP1/ABI3* gene sequence contains intron as buckwheat does, while other three sequences correspond to cDNAs so lack intron sequences. The boxes indicate position of the exons and the conserved splicing sites GT and AG located at the 5'- and 3'-ends of the intron, are indicated by the asterisks above the sequence.

full-length of the *BwVP1* gene, and another PCR reaction was carried out to affirm the presence of the obtained sequence in the buckwheat genome. The primers were designed from the sequence of the 5'- and 3'-TAIL-PCR products and amplified a 3.3-kbp fragment (Fig. 1). The nucleotide sequence was identical to the expected sequence. We analyzed open reading frames in the sequence and determined protein-encoding regions by comparing the intron positions of other *VP1/ABI3* genes, as the VP1/ABI3 homologues contain six exons and five introns at conserved positions among various plant species (Giraudat et al., 1992; Lazarova et al., 2002).

The predicted amino acid sequence of the *BwVP1* gene indicated that it was capable of encoding for a polypeptide composed of 777 amino acid residues with a predicted molecular weight of approximately 84.9 kDa (Fig. 1). Comparison of the deduced amino acid sequence of *BwVP1* to other VP1/ABI3 proteins by multiple-alignment indicated that the homologue of buckwheat contains all four conserved regions (A1, B1–3, Fig. 3). The similarity of these domains within other dicotyledonous plants was A1, 43–53%; B1, 59–71%; B2, 51–72%; B3, 83–92%. The core of B2, RKKR in buckwheat, contains the putative nuclear targeting motif (Chelsky et al., 1989; Lazarova et al., 2002). The amino acid sequence of *BwVP1* was most similar to that of *Arabidopsis thaliana*. The sequence identity was especially high in B3, the putative regulatory domain for transcription.

Southern blot analysis was performed on the genomic DNA, to examine the genomic organization of the *BwVP1* locus in buckwheat, using the PCR product corresponding to the B3 domain as a probe. Under high stringency conditions at 60°C as recommended by the manufacturer, only one band was predicted by the position of the restriction sites in the *BwVP1* gene for each enzyme, but additional bands of varying intensities were also observed (Fig. 4). Some of the extra bands revealed comparable intensities to the expected band in the *EcoRI* digest, and there were also weaker bands in each lane.

DISCUSSION

To isolate a target gene, we generally utilize PCR with degenerate primers designed from amino acid sequences conserved among various species. The accumulation of large amounts of nucleotide sequence data in the data banks also makes these PCR-based strategies easier and more convenient. However, we also have to use other time-consuming methods, such as the construction and screening of cDNA or gDNA libraries, to acquire a full-length sequence of the gene because of an inevitable defect of the PCR with degenerate primers. Conserved regions are usually located inside a protein, so the obtained PCR products often lack a "head-" and a "tail-" portion of the gene. This paper describes the use of thermal asymmetric interlaced PCR (TAIL-PCR) for efficient recovery of the 5'- and 3'-regions flanking the fragment as derived by degenerate primers. TAIL-PCR has been developed to isolate target segments adjacent to known sequences, such as the isolation of promoter regions based on cDNA sequences and mapping of T-DNA transposition sites in the genome (Liu et al., 1995; Smith et al., 1996). This method has advantages over other methods with respect to simplicity, specificity, efficiency, speed and sensitivity. TAIL-PCR requires neither special DNA manipulations before PCR nor laborious screening afterwards. When using a set of nested sequence-specific primers, the reliability of the PCR products also can be confirmed very simply by electrophoresis. These advantages are considered to be preferable in determining the complete sequences of genes in plants possessing largely undetermined genomes, such as buckwheat. Elucidating the 5'- and 3'-regions of a gene may be essential to allow for understanding the precise function and the regulatory mechanisms of the gene, even if those regions did not encode for proteins. It is well known that promoters existing in the 5'-flanking regions play an important role in regulating the transcription of genes (Meshi and Iwabuchi, 1995; Busk and Pages, 1998). As well, various RNA elements in untranslated regions of mRNAs have also been reported to mediate post-transcriptional controls, such as RNA transport, localization, stability, and translation efficiency of the mRNAs (Pesole et al., 2001).

VP1/ABI3 proteins are proposed to be one of key mediators working in various aspects of development and germination in seeds of monocots and dicots (McCarty et al., 1991; Giraudat et al., 1992). Furthermore, the VP1/ABI3 proteins are also closely related to ABA-mediatory phenomena in seeds (Finkelstein et al., 2002). The amino acid sequence as deduced from the buckwheat *VP1/ABI3* gene (*BwVP1*) has indicated that it encodes for a putative polypeptide of 777 amino acid residues with a predicted molecular mass of approximately 84.9 kDa. Both the predicted number of the amino acid residues and the molecular size coincide well with those of other VP1/ABI3 proteins characterized in various plants (McCarty et al., 1991; Giraudat et al., 1992; Lazarova et al., 2002; Nakamura and Toyama, 2001; Footitt et al., 2003). Moreover, the homology of BwVP1 to other VP1/ABI3 proteins at the deduced amino acid sequence level is high in four conserved domains (A1 and B1–B3, Fig. 3). The A1 domain corresponds to an acidic region that acts in activating the transcription of genes, and three B domains are basic regions identified by comparison among the VP1/ABI3 proteins in various plants (Finkelstein et al., 2002; Footitt et al., 2003). The N-terminal B1 and B2 domains are implicated in nuclear localization and interactions with other proteins (Giraudat et al., 1992). Homology

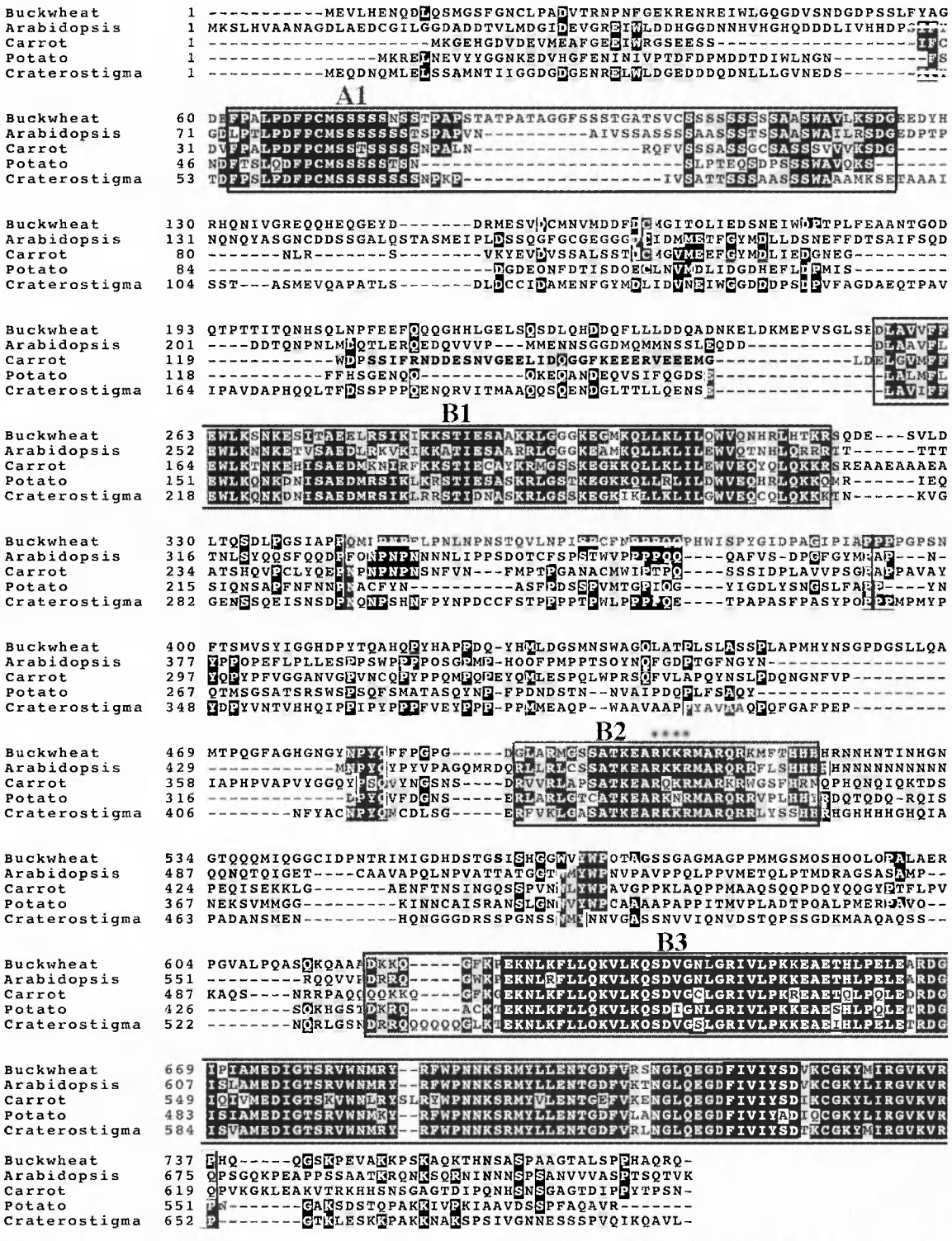

Fig. 3. Alignment of the deduced BwVP1 protein sequence with other previous reported VP1/ABI3 proteins from dicots. VP1/ABI3 proteins [buckwheat (accession no. AB099513), *Arabidopsis thaliana* (X68141), carrot (AB005558), potato (AJ309218), *Craterostigma plantagineum* (AJ000552)] were aligned with the computer programs CLUSTAL W and Boxshade. The four boxes correspond to the previously described regions of highest sequence homology: the acidic A1 region and the three basic regions, B1–3. The putative nuclear targeting signal (RKKR located in the B2 region) is indicated by the solid dots above the sequence.

was highest within the B3 domain functioning as a binding domain to the RY element, a *cis*-acting element present in promoters of many seed-specific genes (Suzuki et al., 1997). These results suggest that the BwVP1 protein possibly functions as a transcription factor in buckwheat seeds.

Southern blot hybridization, with a probe corresponding to the highly-conserved B3 domain of the VP1/ABI3

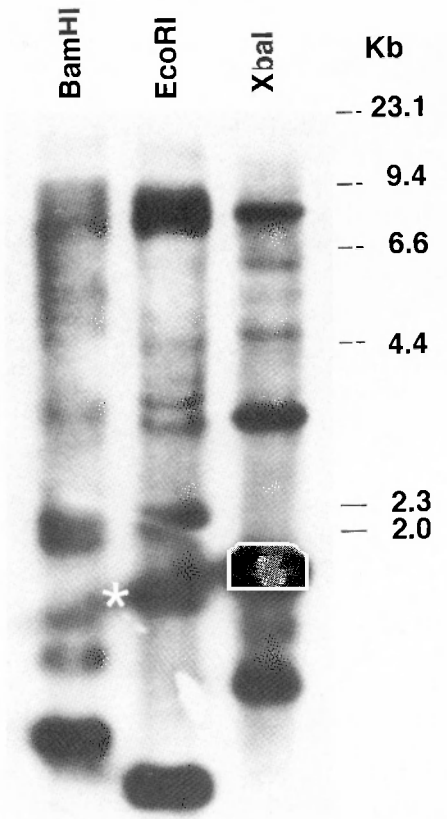

Fig. 4. Genomic Southern blot analysis. Genomic DNA (10 μg) was digested with the restriction enzymes indicated, electrophoresed in a 1.0% agarose gel and transferred onto a nylon membrane. The membrane was hybridized with a fluorescent-labeled fragment corresponding to the B3 domain of the BwVP1 protein. An asterisk in the *EcoRI* lane indicates the predictable band by the position of *EcoRI* restriction sites in the *BwVP1* sequence. The location of predicted bands cannot be shown in the other lanes because of absence of restriction sites corresponding to the position of the probe inside of the *BwVP1* gene.

protein, exhibited multiple bands for DNA samples digested with *BamHI*, *EcoRI* and *XbaI* under high stringency conditions (Fig. 4). Extra bands found in Southern blot analyses have also been observed in yellow-cedar (Lazarova et al., 2002), although it has been shown that there is a single copy of the *VP1/ABI3* gene in genomes of most plants, including *Arabidopsis*, bean and Norway spruce (Giraudat et al., 1992; Bobb et al., 1995; Footitt et al., 2003). Some bands exhibited similar intensities to the predicted bands in the *EcoRI*-treated gDNA, therefore buckwheat allogamy or multiple copies of the *BwVP1* gene might have resulted in these bands. However, other weaker bands might be created by the existence of some *VP1/ABI3*-related genes whose homologies to *VP1/ABI3* genes are relatively low. In fact, some transcription factors, LEAFY COTYLEDON2 (LEC2) and FUSCA3, possess B3 domain and constitute a transcription factor group, the B3 domain family, together with the VP1/ABI3 proteins (Luessen et al., 1998; Stone et al., 2001). We used the fragment corresponding to the B3 domain as a probe, so the extra bands might represent such genes encoding for unknown B3 domain family proteins in buckwheat.

It is well known that the *VP1/ABI3* genes encode for proteins that regulate various aspects of both seed development and ABA responses, and that their mutations can result in vivipary. However, dozens of mutants exhibiting vivipary or defective germination, ex. *abi1-5*, *lec1*, *lec2*, *fus3*, *cho1*, *cho2*, and *rcn1*, have been isolated to date in *Arabidopsis* (reviewed by Finkelstein et al., 2002). The loci causing such phenotypes have been cloned and shown to encode for a variety of proteins, including distinct transcription factors from *VP1/ABI3* proteins. Furthermore, most of the identified genes may also display combinatorial interactions among the many kinds of proteins. Isolation of the *VP1/ABI3* gene is just one of first studies to attempt to shed light on the mechanisms of germination, which is a common but highly complex phenomenon. Further research will be necessary to unravel and finally overcome vivipary.

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Molecular cloning and expression of a major allergenic protein Fag e 2 from buckwheat

Hiroyuki YOSHIOKA, Natsuko YASUEDA and Taiji ADACHI*

Graduate School of Agriculture and Biological Sciences, Osaka Prefecture University, Gakuen-cho 1-1, Sakai, Osaka 599-8531, Japan

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ABSTRACT

It is known that common buckwheat contains several allergenic proteins. Buckwheat allergenic proteins with molecular masses of 9, 14, 16–18, 19, 22–24, 26 and 67–70 kDa have been reported. The N-terminal amino acid sequences of the 16–18 kDa allergenic proteins have already been elucidated. The functions of these allergenic proteins, their pepsin resident, and IgE and IgG binding activities have also been reported. We have isolated and sequenced the cDNA of the 16–18 kDa proteins using N-terminal information. The acquired cDNA coded for 127 amino acids, and the amino acid sequence showed a similarity with the 8 kDa buckwheat allergenic protein, the peanut allergen Ara h 2 and the caster bean allergen Ric c 1 and Ric c 3. We also expressed the recombinant protein using isolated cDNA by means of an *E. coli* expression system. The recombinant protein indicated IgE binding activity which suggests that this protein has potential allergenicity and that the isolated cDNA was identified to code for an allergenic protein.

INTRODUCTION

Common buckwheat (*Fagopyrum esculentum*) belongs to the family Polygonaceae and its seed are used for human food material including noodles, dumplings and tea. Buckwheat is a rich source of vitamins and contains essential amino acids such as lysine, threonine and tryptophan, so that it is becoming popular as a healthy food in many countries. However, buckwheat related hypersensitivity (allergy) is a serious problem among children as well as among adults. Buckwheat seed may cause allergic symptoms for some patients even with a small amount of ingestion. At the present time, the only protection against the buckwheat allergy is to avoid contact with the allergen.

The buckwheat allergy is mainly caused by water-soluble seed storage proteins. Allergenic proteins with a strong IgE binding activity have been identified with a molecular mass of 67–70 kDa, 26 kDa (Urisu et al., 1994), 22 kDa (Nair and Adachi, 1999), 24 kDa, 19 kDa, 16 kDa, 9 kDa (Park et al., 2000), 18 kDa, 14 kDa (Yoshimasu et al., 2000). Among these, the 16 kDa protein was reported as being highly resistant to pepsin digestion (Tanaka et al., 2002), and the 18 kDa protein has an allergenicity of type I and type II, because this protein was recognized with patient's IgE and IgG (Yoshimasu et al., 2000). Interestingly, the N-terminal amino acid sequences of these proteins have complete homology.

In the present study, we isolated the cDNA of the 16–18 kDa proteins using degenerate primers designed from N-terminal amino acids. Antigenicity was confirmed by immunoblotting using a recombinant protein expressed in *E. coli*. We also identified the allergenic protein Fag e 2 for these 16–18 kDa proteins in buckwheat.

MATERIALS AND METHODS

Plant materials

Common buckwheat progeny with BC_6F_2 introgressive generations, between common buckwheat (cv. Miyazaki-zairai) and *F. homotropicum*, was used in the study (Woo and Adachi, 1997). Each individual plant was considered to be of more than 99% of the putative genetic background of *F. esculentum* and expressed a stable self-compatibility system.

cDNA isolation

Total RNA was isolated from young buckwheat seeds 10–15 days after pollination (DAP) according to the CTAB (Cetyltrimethlammonium Bromide) method (Chang et al., 1993). Single stranded cDNAs were synthesized using Hind III/EcoR I/BamH I-oligo dT primer and SuperScriptTMIII Reverse Transcriptase (Invitrogen) according to manufacturer's protocol. Based on the N-terminal amino acid sequence of the 16–18 kDa buckwheat proteins (Tanaka et al., 2002; Park et al., 2000; Yoshimasu et al., 2000), RDEGFDLGETQMSSKCMR, two degenerate primers: BWA1 (5'-MGN GAY GAR GGN TTY GAY YT) and BWA2 (5'-GAY YTN GGN GAR CNC ARA TG) were synthesized (Life Technologies). PCR reactions were performed with 5 µl of template, 100 µM dNTP 20 pmol of *Hind* III/*EcoR* I/*BamH* I

* Corresponding author. E-mail: taijiada@plant.osakafu-u.ac.jp

primer 200 pmol of degenerate primers, 10×PCR buffer and ex*Taq* polymerase (TaKaRa). PCR amplification was followed by denaturation for 2 min at 94°C and 40 cycles of denaturation for 30 sec at 94°C, annealing for 1 min at 50°C and elongation for 1 min at 72°C.

Cloning and sequencing of PCR products

The amplified PCR product was separated by electrophoresis on 1% agrose gel. About 500 bp single band was purified and inserted into pGEM-T Easy Vector (Promega). This plasmid was transformed into *E. coli* strain DH-5α. Positive colonies were screened by blue/white selection. Three positive clones carrying the plasmid with the appropriate insert size of approximately 500 bp were selected for sequencing. The DNA sequence reaction of the selected clones was performed on CEQTMDTCS-Quick Start Kit (BECHMAN COULTER) and sequenced using CEQ2000 (BECHMAN COULTER).

Expression of the recombinant Fag e 2

The plasmid containing the cDNA of the 16–18 kDa protein as a template and the primer sets 15 kDexF (5'-AGAGCTCGAGGATTTTGGTGAAACT 3') and 15kDexR (5'-GAGAGGATCCTTACACAAAATACCG-3') were used for PCR. The 400-bp PCR product was digested with *Bam*H I and *Xho* I and ligated between the *Bam*H I and *Xho* I cloning sites of the pET-15b expression vector (Novagen). This expression vector contains the gene coding for ampicillin resistance and the coding sequence for His-Tag produced at the NH_2-terminus of the recombinant protein. The constructed vector was transformed into a *E. coli* strain BL21 (DE3) pLysS (Novagen) and the expression of the recombinant protein was induced by the addition of isopropyl-β-thiogalactopyranoside (IPTG) at a final concentration of 1 mM in LB liquid medium. After 3 h of continuous shaking at 37°C, the total protein was extracted from the cell lysate, and the recombinant protein was purified using a HisTrap Kit (Amersham Pharmacia Biotech). These proteins were separated by 15% SDS-PAGE and visualized by CBB staining.

Allergen assay of recombinant Fag e 2 by immunoblotting

The recombinant protein was electroblotted onto polyvinylidene difluoride (PVDF) membrane after separation on SDS-PAGE. The membrane was incubated with sera from patients hypersensitive to buckwheat for three hours at room temperature. IgE binding to the recombinant Fag e 2 was detected by monoclonal alkaline phosphatase-conjugated anti-human IgE (Sigma, 1:1000 diluted) as a secondary antibody. The bound antibodies were detected with the chemiluminescent substrate CDP-Star (Amersham Pharmacia Biotech).

RESULTS AND DISCUSSION

Total RNA was extracted from 10–15 DAP buckwheat seeds. RT-PCR was carried out with two degenerate primers BWA1 or BWA 2. A single band of approximately 500 bp was amplified by using only the BWA2 primer (Fig. 1). The PCR product was ligated into pGEM-T Easy Vector and transformed in *E. coli.* Positive clones containing the appropriate insert size were selected for sequencing (Fig. 2). The open reading frame of the isolated cDNA consisted of 366 bp, and the translated polypeptide with 122 putative amino acids was 14.1 kDa and pI 5.4. The decrease in molecular mass could be due to the less amount of glycosylchains The amino acid sequence as predicted from the DNA sequence were used for an homology search with the BLAST database which revealed sequence similarities with the BW8KD buckwheat allergenic protein (Ono et al., 1978), the Ara h 2 peanut allergen (Maleki et al., 2003) and the caster bean allergens Ric c 1 and Ric c 3 (Bashir et al., 1998) (Table 1). The Ara h 2 peanut allergen was a known trypsin inhibitor (Maleki et al., 2003) and the 16 kDa buckwheat allergen was also reported to be a trypsin inhibitor (Park et al., 1997). Such inhibitor proteins and a 2S albumin were classified as allergenic proteins in plants (Shewry et al., 2002). In addition, pathogenesis-related proteins, including inhibitor proteins, were also reported to be allergenic proteins (Hoffmann, 2000). The polypeptide with 122 amino acids has no amino acid homology with the buckwheat major allergenic protein Fag e 1 (Nair and Adachi, 1999). The cystein residues conserved between Fag e 2 and other homological plant allergens suggest that these proteins

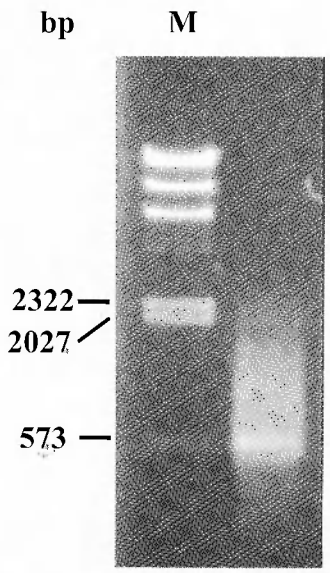

Fig. 1. The amplification of *Fag e 2* cDNA. The RT-PCR product was separated by 1% agarose gel electrophoresis. M: λDNA/*Hin*d III marker.

```
1   GATTTTGGTGAAACTCAGATGAGTTCAAAGTGCATGCGACAAGTGAAGATGAATGAGCCA
      D  F  G  E  T  Q  M  S  S  K  C  M  R  Q  V  K  M  N  E  P    20

61  CATCTGAAGAAATGTAACCGTTACATAGCCATGGATATACTCGATGACAAATACGAAGAA
      H  L  K  K  C  N  R  Y  I  A  M  D  I  L  D  D  K  Y  E  E    40

121 GCTTTGAAGAGGATTGAAGGTGAAGGATGCAAGAGTGAAGAGTCGTGTATGAGAGGATGC
      A  L  K  R  I  E  G  E  G  C  K  S  E  E  S  C  M  R  G  C    60

181 TGTGTGGCGATGAAGGAGATGGATGATGAATGTGTTTGTGAGTGGATGAAGATGATGGTT
      C  V  A  M  K  E  M  D  D  E  C  V  C  E  W  M  K  M  M  V    80

241 GAGAATCAAAAAGGGCGTATTGGTGAAAGGTTGATTAAGGAGGGGGTTAGGGATTTGAAG
      E  N  Q  K  G  R  I  G  E  R  L  I  K  E  G  V  R  D  L  K   100

301 GAATTGCCTGGTAAGTGTGGGCTTAGTGAATTGGAATGTGGATCGAGAGGAAATCGGTAT
      E  L  P  G  K  C  G  L  S  E  L  E  C  G  S  R  G  N  R  Y   120

361 TTTGTGTAATTTGGTTGTTGTGTTGTTGTTTGATGAATAATAAGTAGATGACTTGTTCCC
      F  V  *

421 TCTTGTGGCTATGTATGCTATTGGAGAGGGAAGAAGTTTGTCTGTTGTAAGTTGTTGTGT

481 TAAGCAACTGAAATATATGAGCACTTAACTCTTTAAAAAAAAAAAAAAAAAAAAAAAAAA

541 AAAAGGATCCGAATTCAAAGCTT
```

Fig. 2. Nucleotide sequence of *Fag e 2* cDNA isolated from buckwheat. The nucleotide sequence is on the first line. The second line is derived amino acid sequence. The numbers on the left side indicate the position of the nucleotide sequence relative to the first nucleotide in the insert. The numbers on the right side of figure indicate the position of the amino acid sequence. The asterisk marks the TAA stop codon.

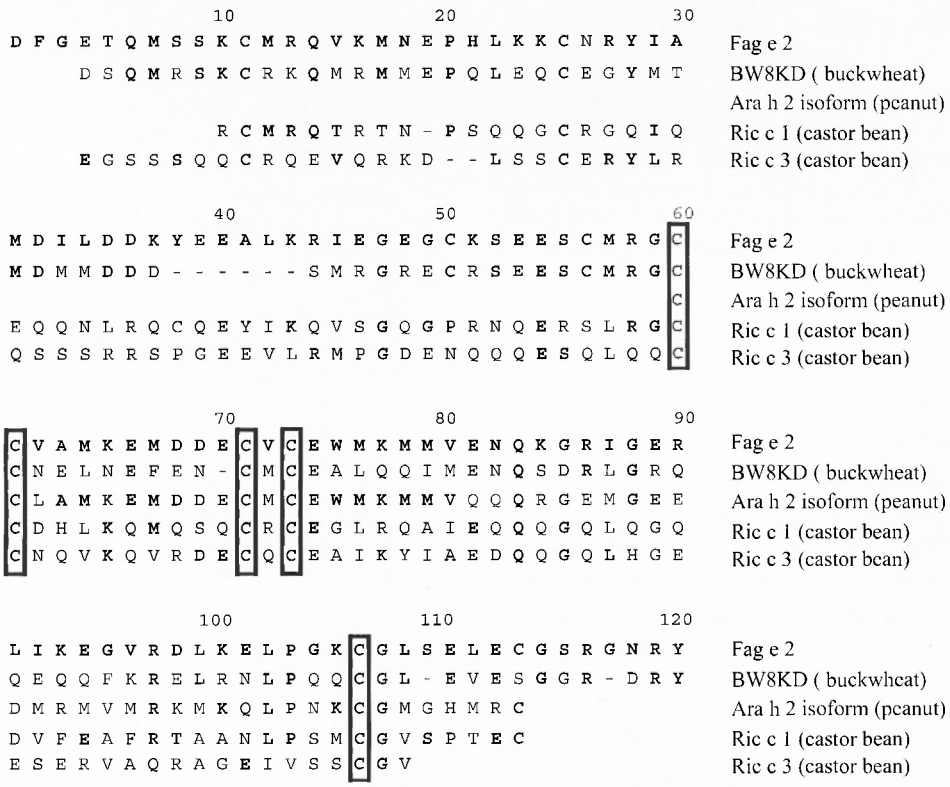

Fig. 3. Alignment of the amino acid sequences between Fag e 2 and other plant allergens. The identical amino acids were bolded, and highly conserved cysteines were boxed.

have a structural similarity related to allergenicity (Fig. 3). The recombinant protein was expressed in *E. coli* and electroblotted onto a PVDF membrane and detected with IgE binding (Fig. 4). These results suggest that the isolated cDNA was coding for an allergenic protein and its linear amino acid sequence was recognized by the patient's IgE. IgE bindings without any recombinant protein were also seen which may be improved by further

Table 1. Amino acid identities between Fag e 2 and allergens. Amino acid identities between allergenic proteins and the predicted protein sequence of Fag e 2. These identities were calculated from multiple sequence alignments.

Species	Name	Amino acid identity (%)
Buckwheat	BW8KD	51
Peanut	Ara h 2 isoform	33
Castor bean	Ric c 1	30
Castor bean	Ric c 3	25

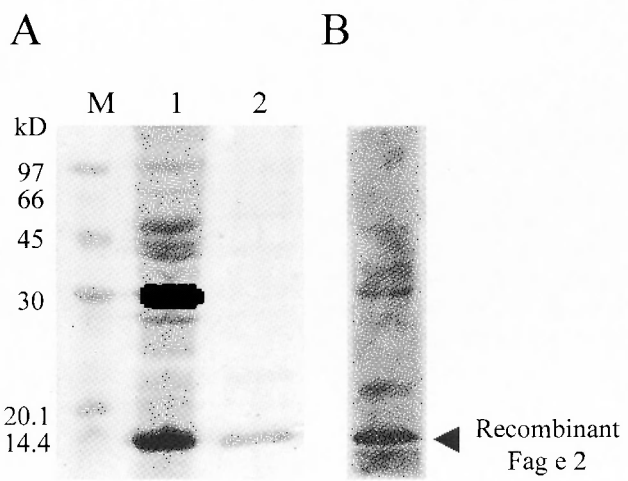

Fig. 4. Expression of Fag e 2 and immunoblotting. A: Expression and purification of recombinant Fag e 2. M: Molecular weight marker, 1: Total protein extracted from cell lysate of expression induced *E. coli*, 2: Purified recombinant protein using Histrap kit. Proteins were separated by 15% SDS-PAGE and visualized by CBB staining. B: Immunoblotting using patient's sera with purified protein.

purification of the recombinant protein. In the present study there was no negative control so we intend to perform additional experiments to support our findings.

This is the first report of cDNA isolation of the 16–18 kDa proteins, and the identification its antigenicity using a recombinant protein, so that we could conclude that this cDNA was coding for Fag e 2. It will be necessary to continue further studies to accumulate knowledge on epitope mapping and other allergen identification which is hoped will lead to successful allergy therapy.

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Quantification of a major allergenic protein in common buckwheat cultivars by an enzyme-linked immunosorbent assay (ELISA)

Wakako MARUYAMA-FUNATSUKI[1]*, Kaien FUJINO[2], Tatsuro SUZUKI[3] and Hideyuki FUNATSUKI[1]

[1] *Department of Low-Temperature Sciences, National Agricultural Research Center for Hokkaido Region, Sapporo, Hokkaido 062-8555, Japan*
[2] *Department of Crop Physiology, Graduate School of Agriculture, Hokkaido University, Sapporo, Hokkaido 060-8589, Japan*
[3] *Department of Upland Agriculture Research, National Agricultural Research Center for Hokkaido Region, Memuro, Kasai-Gun, Hokkaido 082-0071, Japan*

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Key words: allergenic protein contents, BW24KD, common buckwheat, ELISA, quantity variation

ABSTRACT

BW24KD is an important major allergenic protein. There is a large need for buckwheat cultivars lacking the major allergenic proteins. As a first step in the development of these new cultivars, we developed an enzyme-linked immunosorbent assay (ELISA) system that has a demonstarted high performance for quantifying the content of the BW24KD protein. Using this system, we quantified the BW24KD content in forty-three common buckwheat cultivars originating from various areas of Japan and from other countries and found that there were differences in the BW24KD protein content among cultivars and within cultivars, although we found no plants lacking BW24KD.

INTRODUCTION

Common buckwheat (*Fagopyrum esculentum* Moench) is cultivated worldwide, and with the seeds and flour being used for cooking and the plants for forage. However, common buckwheat is known to contain highly allergenic hypersensitive proteins (Smith, 1909; Nakamura and Yamaguchi, 1974/75). Several allergenic proteins in common buckwheat have been characterized (Yano et al., 1989; Kondo et al., 1993; Park et al., 1997; Nair and Adachi et al., 1999; Park et al., 2000; Yoshimasu et al., 2000; Kondo et al., 2001; Tanaka et al., 2002). BW24KD, one of the allergenic proteins found, has been shown to bind to patients' IgEs at a higher frequency and with a stronger activity than those of the other allergenic seed proteins (Kondo et al., 1993, 2001), suggesting that BW24KD is the major allergenic protein in buckwheat. The N-terminal amino acid sequence of BW24KD is similar to that of the 11S or 12S globulins found in several plant species (Kondo et al., 1993, 2001). A legumine-like gene, 'FA02', was recently cloned by Fujino et al. (2001), and was suggested that BW24KD corresponds to the β-subunit of the molecule translated from FA02 (Nagata et al., 2000). Wang et al. (2000) suggested that Tartary buckwheat (*Fagopyrum tataricum*) also has a molecule that is similar to BW24KD.

There is a large demand for buckwheat cultivars lacking any allergenic proteins; however, there have been no reports presenting results of quantitative studies on the allergenic proteins of buckwheat cultivars. It is therefore not known whether the BW24KD content of different cultivars vary genetically. Furthermore, the BW24KD contents of individual plants within a cultivar could vary since buckwheat reproduces in an allogamous manner.

The enzyme-linked immunosorbent assay (ELISA), in which an antibody to the target protein is used, is a powerful tool for the quantification of the target protein and for the screening of a large number of samples (Pollart et al., 1991; Vailes et al., 2001; Palosuo et al., 2002; Randall et al., 2003; Yamashita et al., 2002). An antibody of BW24KD has been developed against the recombinant BW24KD protein by Fujino et al. (2001).

In this study, we report on the development of an ELISA system suitable for the quantification of BW24KD in common buckwheat. Results of the quantification of BW24KD in common buckwheat cultivars originating from various areas of Japan and from other countries are also presented.

MATERIALS AND METHODS

1. Common buckwheat materials

Nineteen breeders' varieties of common buckwheat from Japan, Russia, Canada and China were utilized in the present study. Seeds from 24 local cultivars that were collected in various parts of Japan (see Table 2, Fig. 2)

* Corresponding author. Fax: +81-11-857-9478, E-mail: maruwaka@affrc.go.jp
Abbreviations: BCIP, bromo chloro indolyl phosphate; BSA, bovine serum albumin; CBB, Coomassie brilliant blue; ELISA, enzyme-linked immunosorbent assay; NBT, nitro blue tetrazolium; PBS, phosphate-buffered saline; PVDF, polyvinylidene difluoride; SC, standard curve; S.D, standard deviation; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TBS, Tris-buffered saline.

and in Russia were also used. The local cultivars are thought to have been produced for many years in the regions where they were collected.

Nine grams (approximately 300 grains) of seed of each cultivar was sown and the plants grown in a plot (2.5×2.4 m) in a field located at Memuro, Hokkaido, Japan from June to September in 1998. In order to minimize the outcrossing between the different cultivars, seeds of a tetraploid cultivar, Hokkai No. 3, were planted in an area 2.4 m in width between the plots as described by Funatsuki et al. (2000). Seven to ten individual plants within each cultivar were harvested separately, and the remaining plants were harvested for bulked seed stock of each cultivar.

2. Immunoblot analysis of buckwheat seed proteins with antiserum against BW24KD

The seed proteins of the bulked seeds of all cultivars used in the present study were prepared as follows. One hundred seeds were crushed in a mortar, the hulls were then removed and the remaining endosperms and embryos were ground with a pestle. The seed proteins were extracted from the flour by immersion of the flour in 5 volumes of Tris-buffer consisting of 2% sodium dodecyl sulfate (SDS), 5% 2-mercaptoethanol, 10% glycerol, and 0.0625 M Tris-HCl (pH 6.8) for 10 min at 4°C. Five μl of the seed protein solution was subjected twice to 12.5% SDS-polyacrylamide gel electrophoresis (PAGE). One gel containing the resolved proteins was stained with 0.2% Coomassie brilliant blue (CBB), and the proteins in the other gel were electroblotted onto a polyvinylidene difluoride (PVDF) membrane (Millipore, USA) using an NA-1512 (Nihon-eido Co., Japan). The blotted proteins were blocked with 3% bovine serum albumin (BSA) in Tris-buffered saline (TBS, pH 7.4) at 4°C overnight. An antiserum against BW24KD produced through a rabbit as described by Fujino et al. (2001) was diluted (1:1000) in TBS and utilized as the primary antibody. The antibody was incubated with the membrane at room temperature for one hour with light shaking. After washing with TBS, diluted (1:1000) alkaline phosphatase-conjugated goat anti-rabbit IgG (E.Y. Laboratories, USA) as a secondary antibody was subsequently reacted with the membrane for one hour at room temperature. After washing with TBS, the membrane was soaked in a color developing solution (20 ml of 0.1 M Tris-HCl buffer containing 132 μl of 50 mg/ml nitro blue tetrazolium (NBT), 132 μl of 25 mg/ml bromo chloro indolyl phosphate (BCIP) and 0.1 M sodium chloride, pH 9.5). The reaction was stopped by rinsing the membrane with distilled water.

3. N-terminal amino acid sequencing

The extracted proteins of Kitawasesoba were electrophoresed and electroblotted onto a PVDF membrane, followed by staining with CBB. The protein band migrating with BW24KD and an upper band close to BW24KD (BW24KD') in the SDS-PAGE were cut from the membrane and machine-sequenced by Biologica Co. (Nagoya, Japan) using a PROCISE-cLC (Applied Biosystems, USA).

4. ELISA

Six seeds from each individual plant were ground, and the seed proteins were extracted according to the method described above except that 2-mercaptoethanol was excluded from the Tris-buffer. The protein concentration of the extraction was quantified by using a Bio-Rad *DC* Protein Assay kit (Bio-rad, USA), in which protein concentrations were calibrated to the standard curve (SC) for BSA based on the Lowry assay (Lowry et al., 1951). The extraction was diluted with a coating buffer (0.05 M sodium carbonate-dicarbonate containing 0.1% sodium azide, pH 9.6) to a concentration of 10 μg/ml just before the ELISA analysis.

Ninety-six wells of a microtiter plate were coated with 100 μl of diluted protein extractions. The same plate was coated with recombinant BW24KD expressed in *E. coli* by Fujino et al. (2001) at concentrations of 0.1, 0.2, 0.4, 0.8, 1.0 μg/ml to generate SCs for every plate. Three wells were coated with the protein extraction from each sample. The plates were incubated at 4°C overnight and then washed three times with 200 μl of 0.01 M phosphate-buffered saline (PBS) containing 0.05% Tween 20 (pH 7.4). Two hundred μl of antiserum against BW24KD (diluted to 1:6000) was added to all the wells, and the plate was then incubated for 2 hr at room temperature. After washing with a PBS buffer, alkaline phosphatase-conjugated goat anti-rabbit IgG (1:1000) was added, and the plate was again incubated for 2 hr at room temperature. After the final washing, the signals were developed by the addition of 200 μl of 0.05 M sodium carbonate-dicarbonate buffer containing 1 mg/ml 4-nitrophenol-phosphate and 0.5 mM magnesium chloride (pH 9.6) and the reaction was stopped by the addition of 50 μl of 1 N sodium hydroxide to each well. The absorbance was read at 405 nm. The BW24KD content in each well was determined by adjusting the absorbance to SC produced from the recombinant BW24KD in the same plate. The BW24KD content of each individual is shown as the average of triplicate determinations. An analysis of variance was applied for the BW24KD content of the 43 cultivars.

RESULTS

1. Immunoblot analysis of seed proteins of common buckwheat using an antibody against BW24KD

An antibody developed against recombinant BW24KD

reacted with more than two protein bands of Kitawasesoba (Fig. 1. B). Although BW24KD was shown as one band in the SDS-PAGE (Fig. 1. A), the immunoblot analysis revealed that BW24KD consists of more than one band (Fig. 1. B). The upper band which was close to BW24KD (referred as to BW24KD') was also positive. The N-terminal amino acid sequences of BW24KD and BW24KD' in SDS-PAGE were both GLEQAFXN (X indicating any amino acid residue). The sequence corresponded to the N-terminal amino acid sequence of the possible β-chains of a peptide translated from FA02, being the 378–385th residues of the deduced amino acid sequence. The V8 endoprotease-digested banding patterns of BW24KD and BW24KD' were the same (data not shown), and BW24KD and BW24KD' both reacted with the IgE of a patient having a strong allergic reaction to buckwheat (data not shown). It is therefore thought that the protein bands comprising BW24KD and the additional band, BW24KD', are all translated from FA02 or that parts of them are translated from FA02, while the rest are translated from a very similar gene(s).

Immunoblotting for protein extractions from bulked seed stocks of all other cultivars revealed that the antibody to BW24KD reacted with some bands comigrating with BW24KD and BW24KD' in all cultivars. The results from several cultivars are shown in Fig. 3. These results indicate that the antibody used in this study could specifically detect the major allergenic proteins for all cultivars.

2. BW24KD contents of common buckwheat cultivars

The BW24KD content of common buckwheat cultivars were determined by ELISA. Analysis of variance of the BW24KD content in 43 common buckwheat cultivars revealed that the BW24KD content was significantly different among the cultivars (Table 1). However, the difference in BW24KD content among the cultivars was not very large (Table 2).

In most of the cultivars analyzed in this study, the BW24KD content of individual plants within one cultivar varied greatly (Table 2). The BW24KD content of some individuals were much lower than the means of the BW24KD content in the cultivar, e. g., plants with the lowest content were found in Kitayuki D (0.122 μg in 10 μg of total soluble protein, 1.22%), Aohatakei No. 5 (1.64%), Simokawa (1.70%), Bekkai (1.78%), and Skorospelaya 81 (1.67%). However, no plants that were lacking BW24KD were found.

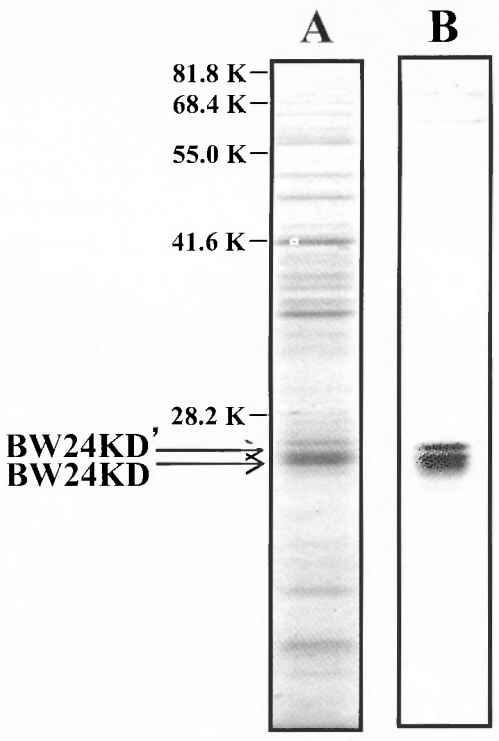

Fig. 1. Immunoblot analysis of seed proteins of Kitawasesoba using an antibody against BW24KD.
(A) Seed proteins of Kitawasesoba were subjected to 12.5% SDS-PAGE and stained with CBB. (B) Seed proteins in (A) were incubated with the antibody to BW24KD (1:1000) and subsequently with an alkaline phosphatase-conjugated goat anti-rabbit IgG (1:1000), and then signals were detected with NBT/BCIP solution. Positive bands are indicated by arrows. Positions of molecular weight markers are shown on the left.

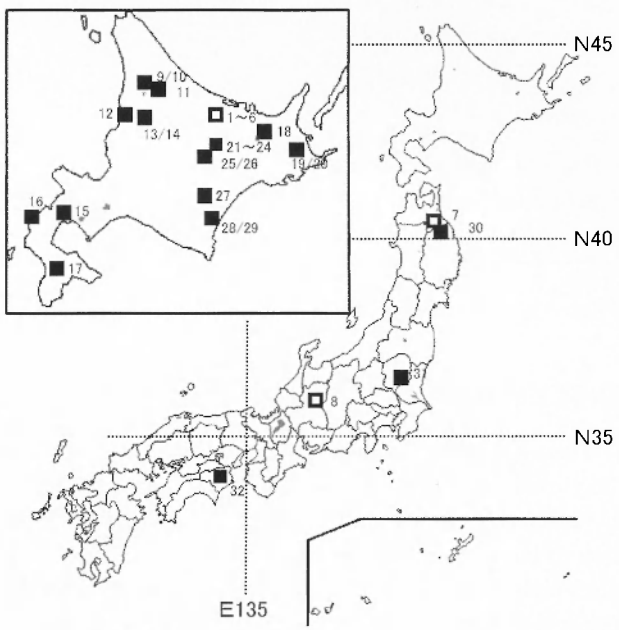

Fig. 2. Map of origins of Japanese common buckwheat cultivars.
The lines show the boundaries of the 47 prefectures in Japan. Hokkaido Prefecture is shown enlarged. Open boxes indicate places in which the breeding was performed for the breeders' varieties, and the close boxes indicated towns or areas in which the local cultivars were collected. The numbers corresponds to those in Table 2.

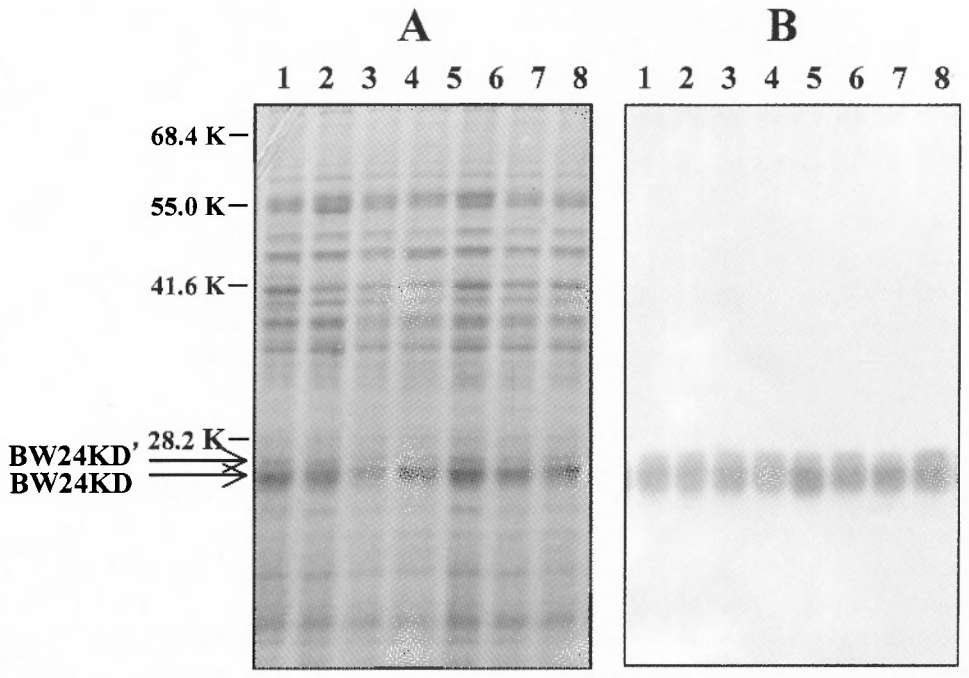

Fig. 3. Immunoblot analysis of seed proteins of common buckwheat cultivars using an antibody against BW24KD.
(A) Seed proteins of local cultivars originating from Kodaira (1), Nayoro (2, 3), Wassamu (4, 5), Setana (6), Atsusawabu (7) and Tyurui (8) were subjected to 12.5% SDS-PAGE and stained with CBB. (B) Seed proteins in (A) were incubated with the antibody to BW24KD (1:1000) and subsequently with an alkaline phosphatase-conjugated goat anti-rabbit IgG (1:1000), and then signals were detected with NBT/BCIP solution. Positive bands are indicated by arrows. Positions of molecular weight markers are shown on the left.

Table 1. Analysis of variance of the BW24KD contents in 43 common buckwheat cultivars

Source of variation	d.f.	Sum of square	Unbiased variance	F-value
Total (T)	424	1.6081		
cultivar (A)	42	0.6255	0.0149	5.7896**
error (E)	382	0.9826	0.0026	

** Significant at 1% level.

DISCUSSION

There have been reported cases in which ingestion of a very small amount of buckwheat has caused allergic hypersensitive reactions (Wieslander, 1996). Identification of buckwheat lines lacking the major allergenic proteins and the development of buckwheat cultivars lacking the major allergenic proteins are therefore very important. The difference in BW24KD content among the *F. esculentum* cultivars in the present study was not large, and no plants were found that lacking the BW24KD protein. It is therefore thought that BW24KD may play an essential biological role in the plant. However, Nair and Adachi (2002) reported that two *Fagopyrum* species, *F. lineare* and *F. urophyllum*, lacked the 22-kDa allergenic protein, of which the deduced amino acid sequence is similar to those of FA02 and FA18. It may therefore be possible to find *F. esculentum* plants that lack BW24KD and grow normally. It is also thought to be difficult to find buckwheat cultivars lacking BW24KD by the screening of bulked seeds of a given population. However, individual plants with very small amounts of BW24KD were found in many cultivars. Some individuals had a BW24KD content similar to or lower than one half of the mean content within the cultivar. If BW24KD and BW24KD' are encoded by FA02 as suggested by the N-terminal amino acid sequencing, individual plants with a very low content of BW24KD might be heterozygous for the defective gene. If so, a cross between the heterozygous plants could produce progenies lacking BW24KD and BW24KD', which may be homozygous for the defective gene. Furthermore, screening of a large number of plants might enable an individual plant lacking either BW24KD nor BW24KD' to be found. This possibility is supported by the fact that a determinate mutant, which is a phenotype derived from a single recessive gene, was found in a population of nine thousand buckwheat plants (Funatsuki et al., 1996). In addition, in order to develop cultivars lacking the major allergenic proteins, development of a null mutant for the gene(s) encoding BW24KD and BW24KD' may also be useful. For this

ERRATUM

Fig. 3 of Maruyama-Funatsuki should be replaced by the following new Fig. 3.

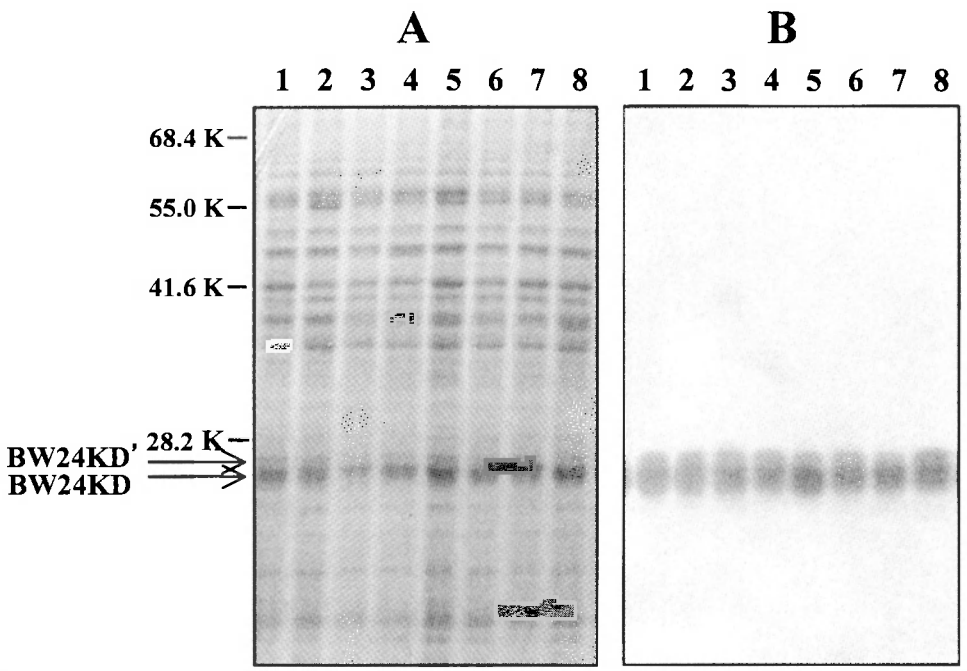

Fig. 3. Immunoblot analysis of seed proteins of common buckwheat cultivars using an antibody against BW24KD.
(A) Seed proteins of local cultivars originating from Kodaira (1), Nayoro (2, 3), Wassamu (4, 5), Setana (6), Atsusawabu (7) and Tyurui (8) were subjected to 12.5% SDS-PAGE and stained with CBB. (B) Seed proteins in (A) were incubated with the antibody to BW24KD (1:1000) and subsequently with an alkaline phosphatase-conjugated goat anti-rabbit IgG (1:1000), and then signals were detected with NBT/BCIP solution. Positive bands are indicated by arrows. Positions of molecular weight markers are shown on the left.

Table 2. BW24KD contents of common buckwheat originating from Japan, Russia, Canada and China[1]

No. in Fig. 2	Cultivar[2]	Origin[3]	Mean (μg/ml)	Min. (μg/ml)	Max. (μg/ml)
1	Kitawasesoba	Japan	0.297	0.204	0.418
2	Kitayuki	Japan	0.295	0.219	0.347
3	Botansoba	Japan	0.248	0.178	0.330
4	Mekei No.14	Japan	0.310	0.196	0.484
5	Kitayuki-D	Japan	0.244	0.122	0.345
6	Hokkai No. 3	Japan	0.315	0.260	0.335
7	Aohatakei No. 5	Japan	0.274	0.164	0.377
8	Shinanonatsusoba	Japan	0.306	0.245	0.509
9	Nayoro (local)	Japan	0.366	0.341	0.389
10	Nayoro (local)	Japan	0.348	0.336	0.352
11	Shimokawa (local)	Japan	0.274	0.170	0.339
12	Kodaira (local)	Japan	0.370	0.327	0.391
13	Wassamu (local)	Japan	0.342	0.310	0.352
14	Wassamu (local)	Japan	0.305	0.274	0.337
15	Kuromatsunai (local)	Japan	0.278	0.195	0.391
16	Setana (local)	Japan	0.305	0.267	0.333
17	Atsusawabu (local)	Japan	0.303	0.276	0.340
18	Tsubetsu (local)	Japan	0.325	0.318	0.329
19	Bekkai (local)	Japan	0.260	0.178	0.342
20	Bekkai (local)	Japan	0.276	0.225	0.349
21	Kamishihoro (local)	Japan	0.293	0.213	0.316
22	Kamishihoro (local)	Japan	0.293	0.281	0.310
23	Kamishihoro (local)	Japan	0.286	0.240	0.322
24	Kamishihoro (local)	Japan	0.314	0.258	0.385
25	Shikaoi (local)	Japan	0.292	0.242	0.396
26	Shikaoi (local)	Japan	0.295	0.276	0.332
27	Makubetsu (local)	Japan	0.324	0.287	0.336
28	Tyurui (local)	Japan	0.303	0.285	0.312
29	Tyurui (local)	Japan	0.312	0.271	0.342
30	Kunohe (local)	Japan	0.342	0.223	0.421
31	Mashiko (local)	Japan	0.340	0.252	0.464
32	Tokushima (local)	Japan	0.317	0.266	0.395
	Skorospelaya 81	Russia	0.323	0.167	0.482
	Skorospelaya 86	Russia	0.337	0.269	0.418
	Krasnostreletskaya	Russia	0.341	0.293	0.425
	Maiskaya	Russia	0.274	0.196	0.322
	Shatilovskaya 5	Russia	0.411	0.314	0.602
	Sumchanka	Russia	0.300	0.230	0.331
	Belorussia (local)	Russia	0.252	0.204	0.336
	CM-17	Canada	0.385	0.314	0.461
	Mancan	Canada	0.421	0.284	0.677
	Pennquad	Canada	0.306	0.237	0.343
	Tianqiao	China	0.321	0.310	0.329

[1] Means of BW24KD contents (μg) in 10 μg soluble protein samples are shown. The maximum and minimum contents among individuals of a cultivar are shown.
[2] For local cultivars, the names of towns or areas in which the local cultivars are grown are shown. Local cultivars with the same name were collected from different places in the area.
[3] Origin indicates the country in which the breeding was performed for the breeders' varieties or the country in which a local cultivar was collected.

purpose, it may be important to enlarge the genetic variation in buckwheat materials using artificial techniques, e.g., exposure to radiation or to carbon-ion beams and chemical treatment before selection.

In the strategies described above, it is necessary to screen a very large number of individual plants. Although analysis of large numbers of plants by SDS-PAGE and immunological experiments would take a long time, the

ELISA method described in this study is thought to have high performance for screening many individual plants.

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Inoculation effect on growth and flavonoid content of Tartary buckwheat in a field experiment

Yun-ping TAO[1], Qing-liang SHI[2], Xiao-xiu ZHANG[2] and Yun-ning ZHOU[2]

[1] *Institute of Agricultural Resources Research, Shanxi Academy of Agricultural Sciences, Taiyuan 030006, China*
[2] *White Agriculture R&D Center, Shanxi Academy of Agricultural Sciences, Taiyuan 030006, China*

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ABSTRACT

A field experiment was carried out in Shanxi, China to determine the effect of different microbial inoculants on the growth and flavonoid content of Tartary buckwheat and to decide the best one to apply for local Tartary buckwheat production. These objectives were addressed by blending inoculants with seeds one day before sowing, sampling at various times, based on phenological phases, and simultaneously measuring plant height, root length, stem width, leaf number, fresh and dry weight, and flavonoid content. Some soil fertility factors were also measured after the experiment. The results indicated that some microbial inoculants applied to Tartary buckwheat production in this experiment effectively increased plant growth, flavonoid content, total flavonoid yield, and increased soil nutrient supply. Considering comprehensively, POLY, MIXTURE, and *Bacillus* spp. were the top three among 10 treatments. It is practicable to apply them for local Tartary buckwheat production.

INTRODUCTION

Tartary buckwheat and its products have aroused great concern and have drawn a great deal of attention in recent years due to its important nutritional composition and medicinal value. Tartary buckwheat can be used as a healthy food or as medicine (Park et al., 2000; Zhao, et al., 2001). Buckwheat grain contains more protein than other crops (Lin, 1994; Lin et al., 2002). However, its medicinal value is of greater importance (Wang et al., 2001). The critical medicinal compositions in buckwheat are the biological flavonoids and fagopyritols, sophoretin and heterosides. These components have the effect of relieving cough, and eliminating phlegm. The rutin content of Tartary buckwheat is higher than that of common buckwheat (Zhao et al., 2001; Kreft et al., 2003). Therefore Tartary buckwheat has become a very important raw material of agricultural products. The present market requires an increased number of Tartary buckwheat products.

Shanxi province is located in the eastern part of the Loess plateau in North China. Buckwheat cultivation has been increasing in this province year by year. There is an urgent requirement to conduct more research to allow for an increase in buckwheat production. Most investigations on buckwheat have focused on genetics, breeding, and cultivation; research on buckwheat fertilization has mostly concentrated on chemical fertilizers and their effect (Hayashi, 2001; Yao et al., 2001; Zhang et al., 2001; Yang et al., 2003). However, fertilizer application sometimes causes lodging and decreases yield. Some research has shown that the application of microorganisms could increase soil nutrient supply and stimulate plant growth (Liu and Deng, 1986; Li and Zhang, 1991; Hao et al., 1991; Ge, 1999). There is little information on the effect of microorganism inoculation on Tartary buckwheat flavonoid content and yield. The purpose of the work reported here was to study the effects of different inoculants on the growth and flavonoid content and yield of Tartary buckwheat in a field experiment.

MATERIALS AND METHODS

1. Plant material and soil

The Tartary buckwheat variety used in this experiment was Yu 6-21. The experiment was carried out at the Research Farm, Shanxi Academy of Agricultural Sciences in 2003. The soil type of the field was sandy loam calcareous cinnamon soil; its basic property was as follows: organic matter 1.736%, total nitrogen 0.124%, alkali-hydrolyzed N 38.1 mg/kg, available P 52.3 mg/kg, available K 78 mg/kg, and pH 7.8.

2. Treatments

In order to study the effects of selected inoculants on the growth and flavonoid content of Tartary buckwheat, 200 g of Tartary buckwheat seed for each plot was treated one day before sowing with 20 ml of different inoculants or a mixture as designed ($>3\times10^8$ colony-forming units (cfu)/ml). The plot size was 2.7 m×2.1 m, the seeds were sown at a spacing of 30 cm between rows, 8 rows per plot, in 4 replications and in a randomized block design. The sowing amount was 20 g/plot (about 2.5 g/row). When sowing, shallow furrows were made and the seeds were broadcasted into the furrows. Seeding was on May

16th, 2003, and harvesting on September 15th, 2003. Only the top part of the plants were harvested. 1 kg/plot of basal manure (N 1.73%, P 1.17%, and K 1.29%) were applied before sowing, and no other fertilizers were applied during the experiment. This experiment had 10 treatments as follows:

1) CK_1 (control); 2) CK_2 (sterilized peat) 3) *Azospirillum brasilense* 4) *Streptomyces jingyangesis* 5) *Bacillus* spp. 6) *Bacillus mucilaginosus* 7) *Bacillus megaterium* 8) Arbuscular mycorrhizal fungi *Glomus mosseae* (AMF *G. mosseae*) 9) MIXTURE (*Azospirillum brasilenser+ Bacillus mucilaginous+Bacillus megaterium*) 10) POLY (A mixed inoculant mainly Diazobacter (screened from local Tartary buckwheat rhizosphere)).

3. Analysis methods

The soil property was determined using normal methods. Flavonoid content was determined by spectrophotometric analysis (Chen, 1998). Rutin, used as standard sample to determine flavonoid content, was obtained from YABAO Pharmaceutical Manufactory, Shanxi, China.

4. Measurement method

Ten plants (5 plants at the harvest stage) from each plot were taken each time as samples, and the morphological characteristics such as plant height, root length, stem width, leaf number, and fresh and dry weight were measured and recorded. The flavonoid content of sample mixture of stems and leaves was determined at 5 growth phases, and the flavonoid content of the grain was determined after harvest. All samples were ground and passed through a 0.16 mm sieve. After the experiment, a soil sample 20 cm in depth was taken from three points in each plot, and the soil organic matter, total nitrogen, alkali- hydrolyzed N, available P, and available K were determined.

RESULTS AND DISCUSSION

1. Effect of different inoculants on plant growth of Tartary buckwheat

1.1 The effect of inoculation on young seedling growth

The Tartary buckwheat seeds germinated uniformly and grew well in all plots. Twenty days after sowing (June 6), the first samples were taken and measured. The results were as shown in Table 1. The results showed that CK_1 had the highest plant, but the lowest stem width, leaf number, and total dry weight, therefore the lowest seedling index (see footnote of Table 1). At the stage of seedling growth, the plants were very small. Some of them were high, and some had long roots, and still others had wide stem. The method to compare the results is to get seedling index, and then to compare. The seedling index shown in Table 1 was the mean value of four replications, and each replication had 10 plants. The increase rate (data not listed) was calculated from the following equation: Increase rate $(\%)=\{(B-A)/A)\}\times 100$, where A stands for the value of CK, and B for the value of treatment. The result shown in Table 1 had not been statistically tested, just a simply comparison. Treatment 4, i.e. *Streptomyces jingyangesis*, gave the best performance at this early growth stage, and was followed by treatments 5 and 9, *Bacillus* spp., and MIXTURE (*Azospirillum brasilense+Bacillus mucilaginous+Bacillus megaterium*).

1.2 The effect of inoculation on other growing stages

During the experimental period, samples were taken at the following stages of vegetative growth (June 25th), initial flowering (July 4th), full flowering (July 29th), and harvest (Sept. 15th), and morphological characteristics and yield were measured. All plots lodged slightly on July 10th after a night of wind and rain, but continued to grow upward subsequently. The results are shown in

Table 1. Effect of different inoculants on young seedling growth of Tartary buckwheat

Treatment No.	Plan height (cm)	Root length (cm)	Stem width (cm)	Leaf number (/plant)	Total dry weight (mg/plant)	Top dry weight (mg/plant)	Root dry weight (mg/plant)	Seedling index*
1	15.2	2.88	0.21	3.95	7.50	6.50	1.00	0.019
2	14.9	2.32	0.24	4.15	8.50	7.50	1.00	0.022
3	14.1	2.27	0.21	4.1	7.50	6.75	1.00	0.020
4	13.8	2.01	0.23	4.35	8.75	7.50	1.25	0.025
5	14.3	2.73	0.24	4.25	8.75	7.50	1.20	0.024
6	13.4	1.88	0.23	4.1	8.00	7.00	1.00	0.022
7	14.0	2.54	0.23	4.0	8.50	7.50	1.00	0.022
8	14.4	2.48	0.23	4.15	8.00	7.00	1.00	0.021
9	13.9	2.57	0.23	4.15	9.90	9.00	0.90	0.024
10	12.7	2.92	0.22	4.25	8.50	7.75	1.00	0.023

*: Seedling index=(Stem width/Plant height+Root dry weight/Top dry weight)×Top dry weight. This index reflects the growing state, the higher the better in most cases.

Tables 2 and 3.

Statistical results of Table 2 at initial flowering stage showed that treatments 4, 6, 7, and 10 of total dry weight and top dry weight were significantly higher than CK_1 at 5% level; and treatments 2, 4, 6, and 10 of root dry weight were significantly higher than CK_1 at 5% level. Statistical results of Table 2 of full flowering stage showed that treatments 4, 5, 6, 8, and 10 of total dry weight, treatments 5, 6, 8, and 10 of top dry weight, all treatments except 7 of root dry weight were significantly higher than CK_1 at 5% level.

At harvest stage (see Table 3), treatments 3, 5, and 10 of dry weight of stems and leaves, and treatments 3, 5, 9, 10 of seed weight were significantly higher than CK_1 and CK_2.

These inoculated microorganisms increased the nutrient supply to the plants or improved soil conditions for the plants through their activities, which include nitrogen fixation or phosphorus release into the plant rhizosphere. Nevertheless, *Streptomyces jingyangesis* was not as effective overall as it was at the seedling stage. An conclusion can be drawn from this phenomenon is that the decision of inoculation strains should be based on the final product being produced. For example, *Streptomyces* should be adopted in Tartary buckwheat sprout production to produce buckwheat tea, but *Azospirillum brasilense*, *Bacillus* spp., MIXTURE, and POLY should be applied for stems, leaves and grain production.

Table 2. The effect of inoculation on plant growth

Growth stage	Treatment No.	Plan height (cm)	Root length (cm)	Stem width (cm)	Leaf number (/plant)	Total dry weight (g/plant)	Top dry weight (g/plant)	Root dry weight (g/plant)
Vegetative Growth	1	52.6	9.1	0.54	6.4	0.14	0.12	0.025
	2	57.3	9.6	0.57	6.5	0.13	0.11	0.025
	3	57	10.6	0.55	6.6	0.14	0.11	0.026
	4	55.0	9.5	0.57	6.6	0.13	0.11	0.024
	5	56.2	9.9	0.56	6.6	0.13	0.10	0.023
	6	57.1	10.2	0.57	6.6	0.14	0.11	0.025
	7	54.5	9.8	0.56	6.6	0.14	0.10	0.027
	8	53.0	10.2	0.64	6.5	0.15	0.13	0.023
	9	59.5	10.3	0.57	6.9	0.15	0.13	0.025
	10	56.2	9	0.56	6.4	0.13	0.11	0.022
Initial flowering	1	79.6	9.3	0.54	8.1	2.83	2.44	0.395
	2	80.5	9.7	0.55	7.7	2.95	2.41	0.545*
	3	77.7	9.8	0.58	7.7	3.10	2.75	0.360
	4	81.7	10.0	0.62	8.0	3.49*	3.02*	0.475*
	5	79.9	9.3	0.59	7.7	3.41	2.95	0.470
	6	76.2	8.9	0.65	7.8	4.00*	3.48*	0.520*
	7	80.9	8.9	0.62	7.8	3.56*	3.12*	0.445
	8	75.4	9.1	0.56	8	3.05	2.69	0.365
	9	75.2	9.2	0.60	8.1	3.12	2.77	0.360
	10	84.5	9.0	0.60	7.8	3.49*	3.01*	0.480*
Full flowering	1	135.6	11.0	0.60	75.9	6.17	5.48	0.690
	2	133.9	11.1	0.59	71.7	6.44	5.60	0.840*
	3	147.3	11.6	0.65	70.4	7.54	6.58	0.970*
	4	145.4	11.6	0.67	76.9	8.25*	7.01	1.240*
	5	147.1	12.1	0.68	75.5	8.13*	7.10*	1.030*
	6	142.9	10.9	0.69	84.1	8.06*	7.21*	0.850*
	7	144.1	10.4	0.65	75.6	7.15	6.41	0.740
	8	154.0	11.4	0.70	73.2	7.90*	7.10*	0.800*
	9	137.6	11.0	0.62	74.7	6.94	6.07	0.870*
	10	138.2	13.1	0.65	84.5	7.99*	7.11*	0.880*

*Significant at 5% level.

2. Inoculation effect on flavonoid content of Tartary buckwheat

The results in Table 4 indicate that the flavonoid content of Tartary buckwheat changed with a change in the grow stage. An overall trend is that the younger the plant, the higher the content. Treatment 7, *Bacillus megaterium*, increased the flavonoid content before the flowering stage. The flavonoid contents of MIXTURE were significantly higher than CK_1 at 5% level at vegetative growth stage, and stems and leaves at harvesting stage, and also grain harvested. The flavonoid contents of POLY were significantly higher than CK_1 at 5% level at seedling stage, and vegetative growth stage, and also grain harvested. Besides, treatments 4, 7, and 8 at seedling stage, treatments 3, 6, and 7 at vegetative growth stage, treatments 2, and 5 of stems and leaves at harvesting stage, and treatments 5, and 7 of grain were also significantly higher at 5% level than CK_1.

There were no significant differences at the stages of initial flowering and full flowering. From the other four groups of results, treatments 7, 9, and 10 gave good results in three sampling times respectively. The flavonoid content of CK_1 and CK_2 at the flowering stage were higher than some other treatments due to slow seedling development.

3. The effect of inoculation on flavonoid yield of Tartary buckwheat

Total flavonoid yield (see Table 5) was calculated from flavonoid content (Table 4) and total dry weight (Table 2 and 3). It can be concluded that treatments 10 gave significantly higher flavonoid yield than CK_1 and CK_2 at all stages. It indicates that Poly had relatively strong effect on flavonoid composition. Except at full flowering stage, treatment 9 also gave significantly better results than CK_1 and CK_2. These treatments increased flavonoid yield by more than 20%. For treatment 9, mixture of *Azospirillum brasilense*, *Bacillus mucilaginous*, *Bacillus megaterium*, the 3 kinds of microorganisms played roles together. The *Azospirillum brasilense* had the effect of

Table 3. The effect of inoculation on Tartary buckwheat growth and yield at the harvest stage

Treatment No.	Plan height (cm)	Stem width (cm)	Dry weight of stems and leaves (g/m^2)	Seed weight (g/m^2)
1	179.2	0.67	343.9	78.1
2	163.9	0.65	343.9	76.9
3	163.9	0.78	402.1*	85.9*
4	173.7	0.77	349.2	80.0
5	176.2	0.77	379.2*	95.6*
6	166.3	0.61	317.5	81.7
7	172.6	0.81	331.6	76.2
8	181.8	0.66	326.3	84.6
9	171	0.73	343.9	87.0*
10	184.8	0.80	359.8*	93.7*

*Significant at 5% level.

Table 4. Flavonoid content of Tartary buckwheat at different growth stages (%)

Treatment No.	Seedling	Vegetative growth	Initial flowering	Full flowering	Stems and leaves at harvesting	Grain
1	3.06	2.76	3.08	2.24	1.42	0.55
2	2.99	3.06	3.12	2.22	1.60*	0.55
3	3.39	3.20*	2.67	1.64	1.49	0.51
4	3.51*	2.78	2.98	1.90	1.31	0.54
5	3.25	2.71	2.84	1.82	1.52*	0.61*
6	3.38	3.14*	2.68	1.66	1.26	0.54
7	3.55*	3.27*	3.07	1.75	1.26	0.59*
8	3.52*	2.58	2.68	1.95	1.30	0.56
9	2.96	3.32*	2.97	1.87	1.61*	0.61*
10	3.45*	2.98*	3.13	1.92	1.46	0.59*

* significant at 5% level.

nitrogen fixation, *Bacillus mucilaginous* could secrete acidic material, and *Bacillus megaterium* could help release phosphorus in the soil, all this contributed to get the results. The principal functions of treatment 10, POLY, were nitrogen fixation and improvement of soil conditions. POLY was the only one inoculant screened from local Tartary buckwheat rhizosphere; it was most adaptive to local soil conditions. In addition, treatments 4, 5, and 8 at the stage of seedling growth, treatments 3, 6, and 7 at the stage of vegetative growth, treatments 4, and 7 at the stage of initial flowering, treatments 4, and 8 at the stage of full flowering, and treatment 5 at the stage of harvesting, both stems and leaves and grain, were significantly higher than CK_1.

4. The effect of inoculation on soil properties after experiment

Soil samples were taken after harvesting and were analyzed (see Table 6). The results showed that inoculating microorganisms influenced the soil nutritional content though soil nutrients which were absorbed by plant. The available P content decreased rapidly for all treatments. Table 6 showed that treatment 3 had the best effect on soil organic matter content, soil total nitrogen, and available potassium; treatment 5 had the best effect on soil available phosphorus; and treatment 9 (MIXTURE) had the best effect on soil alkali-hydrolysable nitrogen. Treatment 10, POLY, had a very good overall effect on soil chemical properties in increasing the soil content of total nitrogen, alkali-hydrolysable nitrogen, available phosphorus and available potassium by 10.27%, 10.08%, 17.59%, and 91.43% compared with CK_1. It suggests why MIXTURE and POLY increased the yield of Tartary buckwheat grain and flavonoid from other aspects. The main functions of the microorganisms were that they played roles through symbiotrophism, transferring nutrient to the host plants, or changing soil environment in the rhizosphere.

Table 5. Flavonoid yield of Tartary buckwheat at different growth stage

Treatment	Seedling (mg/plant)	Vegetative growing (mg/plant)	Initial flowering (mg/plant)	Full flowering (mg/plant)	Stems and leaves at harvesting (mg/plant)	Grain (mg/m^2)
1	0.23	31.1	87.4	138.8	250.8	432.1
2	0.25	34.3	92.2	143.5	270.9	429.4
3	0.25	36.1*	82.9	124.0	285.9	443.2
4	0.31*	34.5	104.2*	157.1*	264.8	434.1
5	0.28*	32.6	97.1	148.7	331.5*	591.9*
6	0.27	37.1*	97.7	134.1	220.8	447.9
7	0.3	37.6*	109.8*	125.8	242.3	455.2
8	0.28*	34.7	81.8	154.3*	208.0	478.9
9	0.29*	41.4*	93.1	130.0	308.0*	538.0*
10	0.29*	36.5*	109.3*	154.0*	305.2*	553.6*

* Significant at 5% level.

Table 6. The effect on soil chemical properties

Treatment	OM (%)	Total N (%)	Alka-N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)
1	1.39	0.0926	62	11.09	70.13
2	1.27	0.0977	62	12.46	128.15*
3	1.39	0.1078	62	9.10	140.18*
4	1.24	0.0932	57	10.19	74.33
5	1.32	0.0882	66	19.11**	115.78*
6	1.29	0.0894	65	9.13	96.29
7	1.30	0.0945	63	9.15	81.53
8	1.33	0.1041	63	10.76	130.23*
9	1.14	0.0989	76*	14.47*	117.35*
10	1.35	0.1021	68*	13.04*	134.26*

*, **: Significant at 5% and 1% level, respectively.

CONCLUSION

1. The practice of inoculating Tartary buckwheat seeds before sowing can promote seedling growth, increased grain yield, and flavonoid content and yield.
2. *Streptomyces jingyangesis* increased seedling growth at the early growing stage, therefore it can be utilized in Tartary buckwheat green tea cultivation.
3. *Bacillus* spp., MIXTURE, and POLY increased the flavonoid content and grain yield of Tartary buckwheat. This experiment suggested that under the circumstances of the present trial, *Bacillus* spp., MIXTURE, and POLY can be applied for local Tartary buckwheat cultivation.
4. MIXTURE and POLY had a positive effect on soil fertility.
5. Further detailed and systematical studies about the activity of each inoculant and the combined effects should be conducted in the future.

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Relationship between antioxidant activity and flour and hull color in Tartary buckwheat

Kaori FUJITA[1*], Naoto INOUE[1], Shoji HAGIWARA[2], Zhongfa YANG[1], Masakazu KATO[1] and Motoyuki HAGIWARA[1]

[1] *Faculty of Agriculture, Shinshu University, Minamiminowa, Nagano, 399-4598 Japan*
[2] *National Food Research Institute, Tsukuba, Ibaraki, 305-8642 Japan*

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ABSTRACT

Varietal differences of hull and flour color in Tartary buckwheat were observed among 25 Asian and European local varieties, and the relationship between the color property and antioxidant activity of the flour was analyzed. The color property of the flour and hull samples was determined by the $L^*a^*b^*$ system using a colorimeter. The antioxidant activity of flour was measured by micro-chemiluminescence from a mixed solution of the flour sample, H_2O_2 and acetaldehyde. The b^* and C^* values of the flour correlated positively with the antioxidant activity of the flour ($p<0.002$). On the contrary, the b^* and C^* values of the hull correlated negatively with the antioxidant activity of the flour ($p<0.05$). The significant correlation between the b^* value of the flour that indicates color direction from yellow to blue and the antioxidant activity of the flour suggested that the amount of polyphenolic compounds, such as flavonoids, are related to the varietal difference of the antioxidant activity in Tartary buckwheat flour.

INTRODUCTION

Food color is a cultural symbol which is shared by people that belong to the same ethnic group and also influences the quality of the food (Takamiya, 2004). For example, yellow has been an eternal and noble symbol among the Asian countries from ancient times. In studies on flour obtained from crops, many researchers have focused on the color, as the content of the pigments in the flour, such as some flavonoids and carotenoids, usually affects the flour color and also influences the food grade (Havsteen, 2002; Chu et al., 2000; Osawa et al., 1994). In common buckwheat flour, used to produce soba noodles in Japan, the greenish flour is often preferred for its freshness and fragrance (Ujihara, 2004). Therefore, the color of common buckwheat flour is an important grading factor in its evaluation.

There are a few reports on the color of the flour or seed of Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) (Ikeda et al., 2003; Bonafaccia et al., 2003). Ikeda et al. (2003) analyzed the relationship among flour color, mechanical traits and chemical traits of dough in common and Tartary buckwheat, and found a marked difference in color, i.e., a^*, L^* and ΔE values of between Tartary buckwheat and common buckwheat.

The flour color is often influenced by its polyphenolic content. Therefore, information for flour color may also provide information on its chemical composition (Akiyama et al., 2001). The flour of Tartary buckwheat has a higher polyphenol content than that of common buckwheat and of any other crop (Minghe and Fukang, 1998). This is especially true of the rutin content of Tartary buckwheat flour which is 10 to 100 times higher than that of common buckwheat (Fujita et al., 2003; Wang et al., 1995).

The polyphenolic content not only affects the color but also the antioxidant activity, which has important properties as a functional food for human health. Several researchers have reported on the antioxidative properties of common buckwheat (Zadernowski, et al., 1992; Oomah et al., 1996; Holasova et al., 2002; Watanabe et al., 1995). Watanabe et al. (1997) and Watanebe (1998) isolated 4 catechins and rutin from the groats and identified 8 fractions in the ethanolic extract of the hull in common buckwheat. They also pointed out that the antioxidant activity of catechins was higher than that of rutin.

We have previously reported on the significant varietal differences in the antioxidant activity of Tartary buckwheat landraces from Asian and European countries (Fujita et al., 2003). We therefore expected that there would be a varietal difference in the color of the flour or hull corresponding to the varietal difference in the antioxidant activity of flour, and that the flour color could be a convenient indicator of the antioxidant activity of flour. However, the varietal difference in the color of flour or hull and the relationship between hull or flour color and the antioxidant activity of the flour has not been reported. In the present paper, we investigated the varietal differ-

* Corresponding author

ence of the hull and flour color in Tartary buckwheat and clarified the relationship between the color properties of the flour or hull and the antioxidant activity of the flour.

MATERIALS AND METHODS

Buckwheat flour samples

Twenty-five landraces of Tartary buckwheat that had been collected by and stored at Shinshu and Kyoto Universities, including 13 Nepalese, nine Chinese, two Bhutanese, one Slovenian landraces, and an additional Japanese line (Table 1) were used. A Japanese line, Hokuriku No. 4, which was recently developed by the Hokuriku Research Center, National Agricultural Research Center in Japan, was selected as the standard to compare with the landraces. The altitude of collection sites of the landraces varied from 700 m in Nepal to 3,880 m in Tibet, having a altitude difference of 2,200 m (Table 1). These landraces were chosen from the results of the field experiments for having a similar maturity date, high lodging resistance and high yielding ability. In addition to their agricultural traits, a wide variance of hull color was taken into consideration when we chose the materials for this experiment (Fig. 1).

Table 1. Plant materials and the data of collection site

ID No.	Accsesion No. or line name	Collection site	
		Country	Altitude (m)
1	C92-26	China	3880
2	B9126	Bhutan	3070
3	IR-85-9131-C	Nepal	3700
4	Col# 36	Nepal	3400
5	Col# 45	Nepal	3400
6	N 8629	Nepal	700
7	N 8630	Nepal	1300
8	C 9048	China	1100
9	IR-85-6378-B	Nepal	1140
10	IR-85-6224-B	Nepal	1360
11	C 92-18	China	1620
12	C 92-19	China	1620
13	C 9042 (black)	China	–
14	MY-2-6	Nepal	2250
15	N8629	Nepal	–
16	B 9125	Bhutan	2530
17	C 9126	China	–
18	C 9042 (white)	China	–
19	U-8004 (S)	Nepal	2350
20	IR-85-7-B	Nepal	1805
21	C 9039	China	–
22	C 05-60A	China	–
23	IR-85-9135-B	Nepal	3700
24	#233	Slovenia	–
25	IR-85-9009-B	Nepal	2800
26	Hokuriku No. 4†	–	–

†: Standard line.

The landraces and the standard line were grown in an experimental field at Chushin Agricultural Experiment Station, Nagano Prefecture (36°N, 740 m above sea level) in 2000. The sowing time was 14th July and the harvest time was 19th, 24th, 26th Oct. 2000. The experimental plots were arranged in a randomized block design. Planting density was 40 plants m^{-2}. The amount of fertilizer applied was as follows; N; 250 g m^{-2}, P_2O_5; 250 g m^{-2}, K_2O; 200 g m^{-2} and dolomite; 10,000 g m^{-2}, respectively. The field soil was classified as Andosol by the FAO/UNESCO system and the soil texture was a fine sandy loam according to the international system.

Fully ripened seeds were harvested approximately 100 days after seeding, and were stored in a cooling box at 5°C. After removing the hull by using a food-processing mill, FM-33 (San Co. Ltd., China), all of the groat portion was milled using a high-speed centrifugal mill, P-14

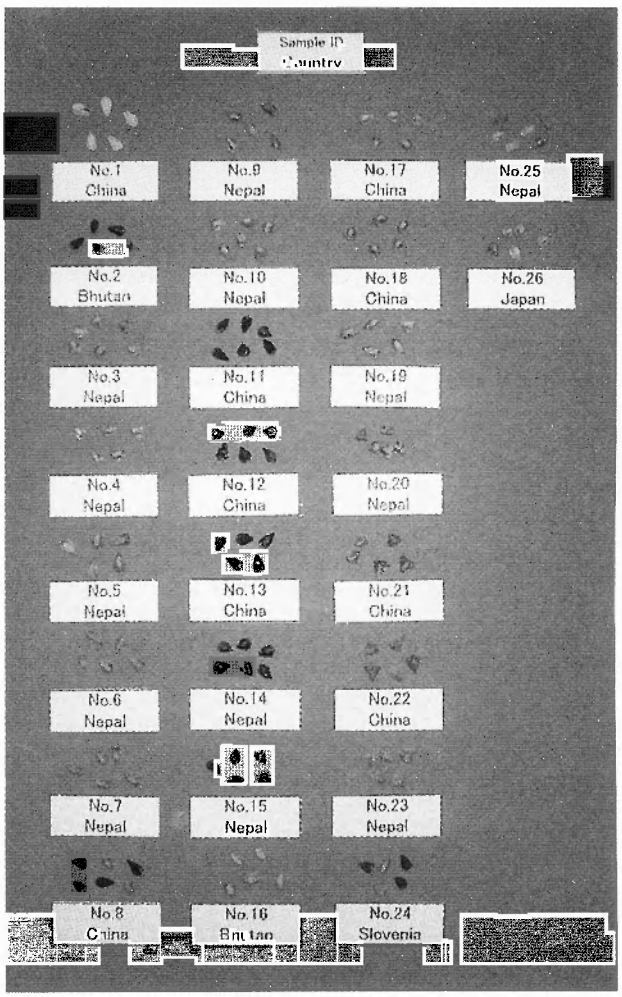

Fig. 1. Experimental materials of Tartary buckwheat.

(Fritsch Japan Co., Ltd., Japan) and was then passed through a 0.5 mm screen to ensure particles uniformity.

Antioxidant activity measurement

The antioxidant activity was estimated by chemiluminescence (CL) intensity. The CL intensity was measured on a mixed solution system: XYZ system (Yoshiki et al., 1995; Akiyama et al., 2001), using micro-photon counter, Aquacosmos/VIM (Hamamatsu Photonics Co. Ltd., Japan).

The measured CL intensity was converted to a relative value of CL from 1 g of Tartary buckwheat flour to that from 10^{-6} M gallic acid (relative CL: RCL) so that the results could be compared with results of other reports. The RCLs are presente in Table 2. The XYZ system and the details of the analysis have been described previously (Fujita et al., 2003).

Color measurement

In this study, the flour and hull colors were expressed in terms of the L*a*b* system, measured with a colorimeter; ZE-2000 (Nippon Denshoku Ind. Co., Ltd., Japan), with the measurement being repeated three times for each sample. The instrument was calibrated with a white ceramic calibration tile (Nippon denshoku Co., Ltd.). The L*a*b* model is an international standard for color measurement developed by the Commission International de l'Eclairage (CIE) in 1976. The lightness and hue are determined by the L*, a*, b* values of the CIE model. In this coordinate system, the L* value is a measure of brightness, ranging from 0 (black) to100 (white), the a* value ranges from −60 (green) to +60 (red), and the b* value ranges from −60 (blue) to +60 (yellow). The derived color function of C* [chroma=$((a^*)^2+(b^*)^2)^{1/2}$] then calculated.

Statistical analysis

Analysis of variance (ANOVA) and correlation analysis were preformed using a personal computer using the programs Excel (Microsoft Co., USA) and Excel Statistics (Esumi Co. Ltd., Japan).

RESULTS

Differences in flour and hull color

Fig. 2 shows the frequency distribution of the color properties of the flour and hull in the landraces examined. From the results of the analysis of variance (ANOVA), all of the color properties measured for the flour and hull were found to be highly significant at a level of significance of less than 0.001 among the 26 Tartary buckwheat cultivars. The L*, a*, b* and C* values of the flour ranged

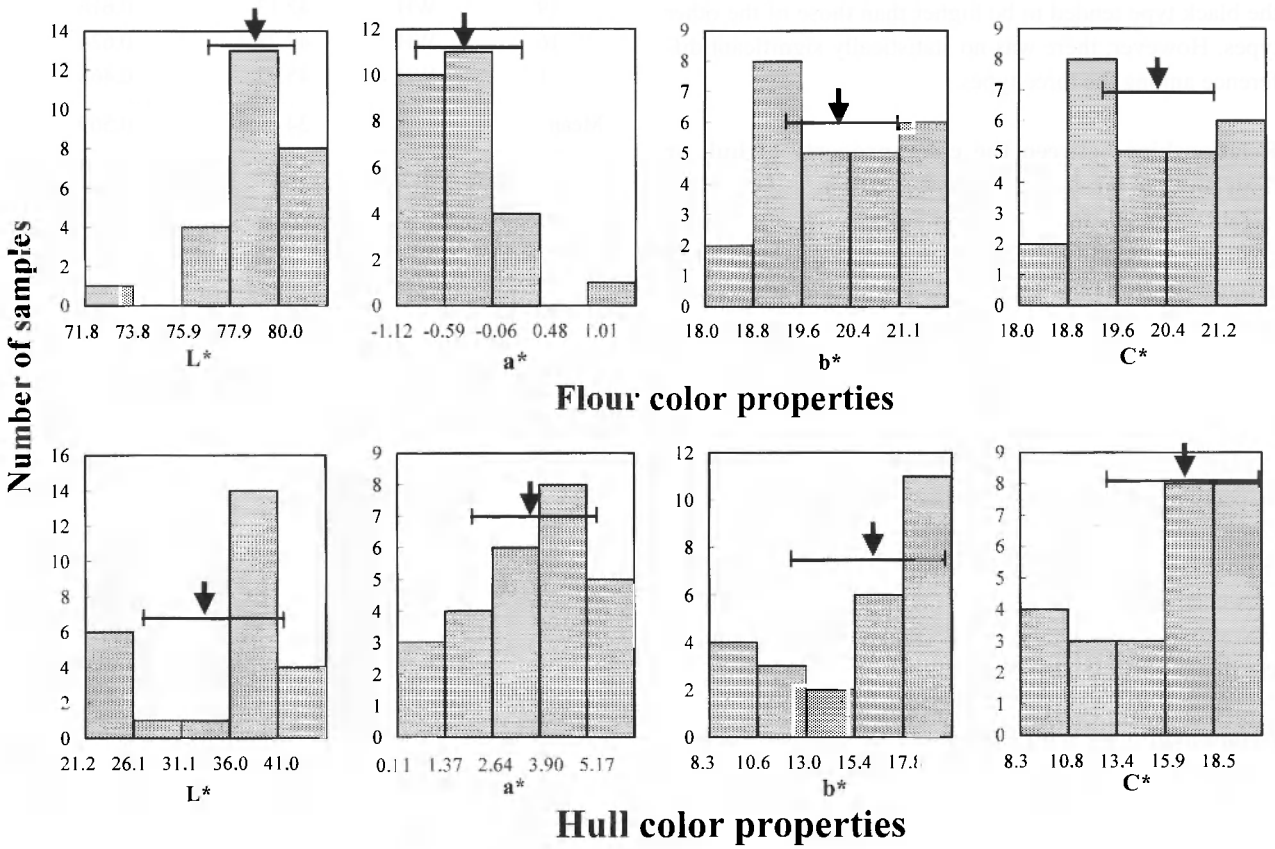

Fig. 2. Frequency distribution of flour and hull color properties.
Arrows and bars indicate average and standard deviation, respectively.

from 71.78 to 82.00, −1.12 to 1.54, 18.02 to 21.91 and 18.02 to 21.94, respectively (Fig. 2). On the other hand, the values of L^*, a^*, b^* and C^* of the hull ranged from 21.15 to 45.92, 0.11 to 6.43, 8.26 to 20.14 and 8.26 to 21.02, respectively. The average values of L^*, a^*, b^* and C^* of the flour were 79.17, −0.40, 20.08 and 20.07, and those of the hull were 34.79, 3.59, 15.78 and 16.21, respectively. The ratio of the maximum value to minimum value in L^*, a^*, b^* and C^* values of the flour were 1.14, 2.38, 1.22 and 1.22, respectively, and those of the hull color, were 2.17, 58.45, 2.44 and 2.54, respectively. The ratio of the maximum value to minimum value in L^*, a^*, b^* and C^* were larger in the hull than in the flour. Negative values were often observed in the a^* value of the flour.

Antioxidant activity in flour from different hull-color types of buckwheat

The hull color type, i.e., black, brown or white was approximated by lightness (L^*). The seed samples were classified into three types based on the L^* value of hull color: black type ($L^*<30$), brown type ($30\leq L^*<40$) and white type ($40.0\leq L^*$) (Table 2).

Fig. 3 shows the antioxidant activity of each hull-color type. The average values of the antioxidant activity in terms of RCL in the black, brown and white types were 0.718, 0.508 and 0.522, respectively. The RCL value of the black type tended to be higher than those of the other types. However, there was no statistically significant difference among the three types.

Relationships between the color property of hull or flour and the antioxidant activity of flour

Table 3 shows the correlation coefficients between the color properties of the hull or flour and the antioxidant activity of the flour. There were significant correlations between the antioxidant activity of the flour and b^* or C^* in the hull or flour. In the flour, antioxidant activity was positively correlated with b^* and with C^* at a highly significant level, ($P<0.002$), while in the hull, the antioxidant activity was correlated negatively with b^* and C^*, respectively ($P<0.05$). Fig. 4 shows the details of these significant correlations. The relationships between the antioxidant activity of the flour and b^* or C^* of the flour is presented in the upper graphs and those of the hull in the lower graphs. More significant correlations were found in the flour than in the hull.

Relationships between the color properties of the hull or flour and the altitudes of seed collection sites

Table 4 shows the correlation coefficients between the altitudes of the seed collection sites and the color properties of the flour or hull. There was no significant correlation between the altitudes of seed collection sites and L^*,

Table 2. Hull color and the antioxidant activity of flour

Sample ID No.	Type of hull color*	Hull color (L^*)	Antioxidant activity in terms of RCL
11	BL	21.15	0.524
15	BL	22.69	0.919
12	BL	23.20	0.528
13	BL	23.58	0.692
2	BL	23.91	0.466
14	BL	24.46	0.948
8	BL	26.60	0.948
25	BR	33.13	0.335
22	BR	36.40	0.380
18	BR	36.56	0.538
24	BR	36.76	0.311
9	BR	36.85	0.446
5	BR	37.06	0.357
21	BR	37.31	0.805
3	BR	37.44	0.330
6	BR	37.62	0.744
7	BR	37.62	0.950
23	BR	38.11	0.388
26	BR	40.05	0.512
20	WH	40.08	0.326
17	WH	40.81	0.532
4	WH	40.96	0.424
10	WH	41.84	0.673
19	WH	42.13	0.616
16	WH	42.23	0.624
1	WH	45.92	0.468
Mean		34.79	0.569
Standard deviation		7.231	0.209

*; Hull color was classified according to L^* as follows:
BL: Black ($L^*<30.0$),
BR: Blown ($30.0<L^*<40.0$),
WH: White ($40.0<L^*$).

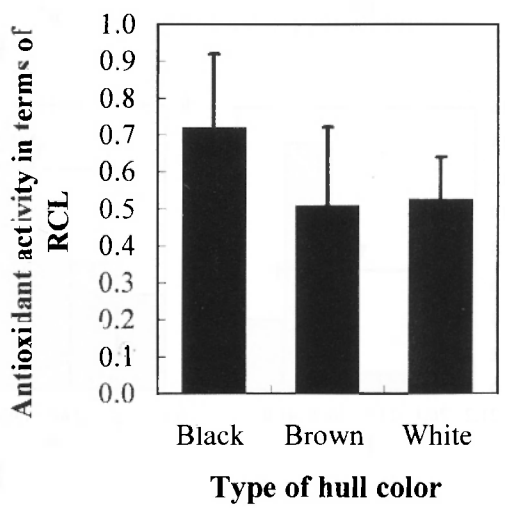

Fig. 3. Antioxidant activity of flour from each hull-color type
Bars in the figure indicate standard deviation.

Table 3. Correlation coefficients between the antioxidant activity of flour and the color properties of flour or hull

	L*	a*	b*	C*
Flour color	0.154	0.370	0.598††	0.606††
Hull color	0.338	−0.360	−0.396†	−0.397†

†; $p<0.05$, ††; $p<0.002$.

Table 4. Correlation coefficients between the altitude of seed collection site and the color property of flour or hull

	L*	a*	b*	C*
Flour color	−0.183	0.289	−0.356	−0.358
Hull color	0.279	0.151	0.142	0.142

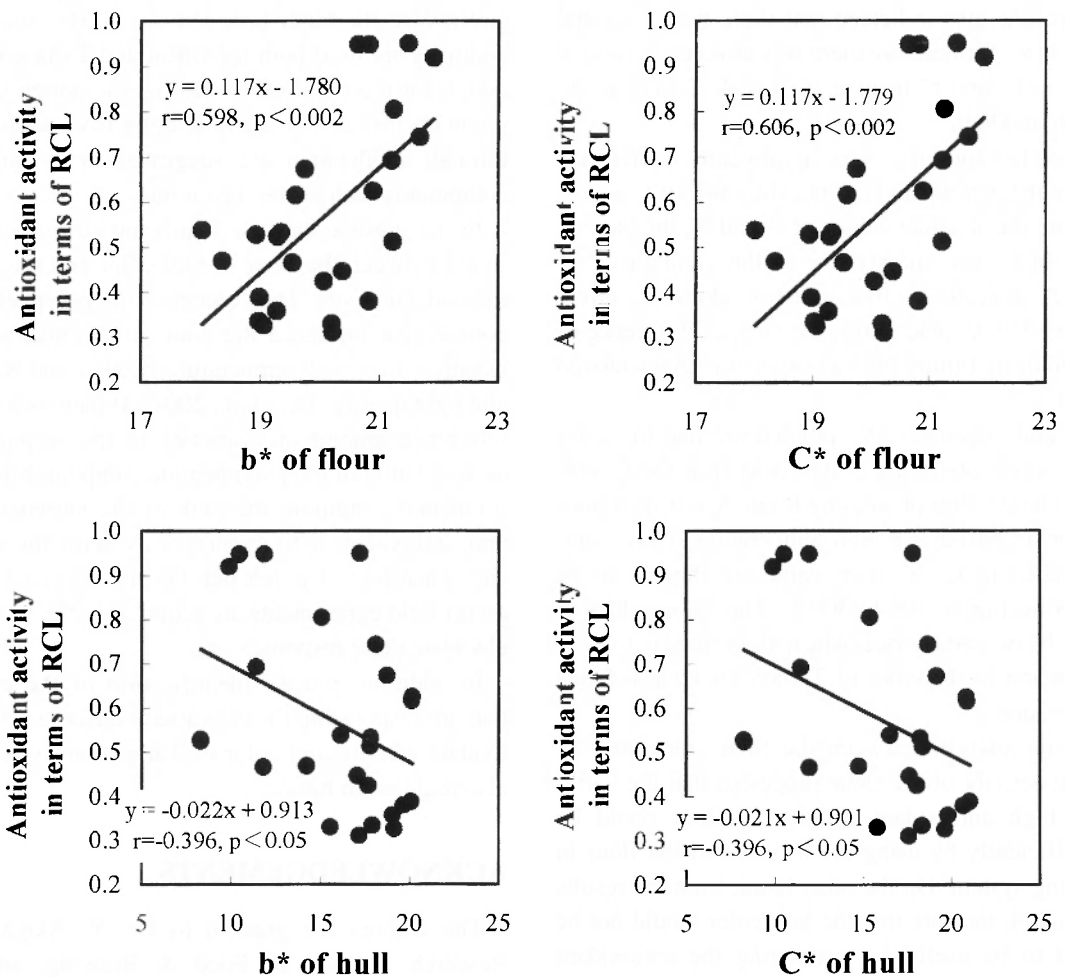

Fig. 4. Relationships between the antioxidant activity of flour and the flour or hull color.

a*, b* or C* in either the hull or the flour.

DISCUSSION

Common buckwheat noodle producers in Japan often evaluate buckwheat flour based on its greenish color which they correlate with freshness and fragrance (Ujihara, 2004). The reason they pay such close attention to the flour color is due to the color of endodermis changing from green to brown during seed ripening in the field and in storage after harvest. On the other hand, consumers of Tartary buckwheat in the southern part of China often prefer a bright-yellow flour (Inoue, 2004). Consumers in Japan, China and other countries have become increasingly interested in Tartary buckwheat flour for its function of improving human health. Tartary buckwheat varieties with a higher human health- keeping function and a more acceptable flour color will be desired in the future. Information on the relationship between the color of flour and its function as healthy food can be very useful for buckwheat breeding programs but is not yet well known. This paper is the first known report describing the varietal difference of the flour and hull color and the corresponding varietal difference in antioxidant activity of flour and the relationship among these characteristics.

The varietal differences found in all the color characteristics, i.e., L*, a*, b* and C* were statistically significant (the results of ANOVA not shown) and the varietal dif-

ference for color was larger in the hull than that of the flour (Fig. 2). In flour, the range of L^* (brightness of color) was the largest, and the range of a^* was the smallest (Fig. 2). Ikeda et al. (2003) reported that the a^* value of Tartary buckwheat dough was markedly higher than that of common buckwheat dough when comparing two common to four Tartary buckwheat varieties. In addition, their data suggested that there was a varietal difference in a^* of the dough among the four Tartary buckwheat varieties. Our results also indicated that there was a varietal difference in a^*, furthermore there was also larger varietal difference in L^* and b^* than in a^* of both Tartary buckwheat flour and hull.

The absolute value of b^* was significantly higher than that of a^* in the present study. Since the value of C^* is calculated from the absolute values of a^* and b^*, the varietal difference in C^* was mainly due to the variation of b^* value which indicates a color variation along the direction from yellow to blue. Thus, the varietal difference of the flour color in Tartary buckwheat is also characterized by b^* and L^*.

Minghe and Fukang (1998) pointed out that the color and flavonoids content of Tartary buckwheat foods were not easily altered after processing it into tea, instant noodles and bread baked at a high temperature. Thus, compounds related to L^*, a^* or b^* value are thought to be stable after heating to 100 to 300°C. The color values of L^*, a^* and b^* of Tartary buckwheat flour appear to be a useful indicator of the color of Tartary buckwheat foods after processing.

Correlation analysis between the flour color and the antioxidant activity of the flour suggested that the varieties with high antioxidant activity of flour could be selected efficiently by using the b^* value of the flour in any breeding system. On the other hand, from the results of Fig. 3 and 4, indicate that the hull color would not be considered to be useful for estimating the antioxidant activity of the flour.

In common buckwheat, the polyphenolics in the groats might be an important factor that determine their color properties (Ikeda et al., 2001), and i.e. rutin, quercetin, cyanidin, catechin and caffeic acid have been identified (Zadernowski, et al., 1992; 21, Luthar, 1992; Watanabe et al., 1998). On the other hand, Tartary buckwheat has an abundance of polyphenolic compounds, such as flavonoids, catechins (Hangels, et al., 1995), vitamin P which have a yellow color (Minghe and Fukang, 1998). This suggest that the varietal difference of the flour color in Tartary buckwheat may be caused by the difference in the content of the polyphenolics which have a yellow color.

In some eastern Asian countries such as, Japan and China, people often add some additives when preparing wheat dough. The most common of additive, called Kansui in Japan, mainly contains potassium carbonate, sodium carbonate and phosphoric salt (The Wheat Society of Japan, 1981). This additive changes the color of the wheat dough from white to yellow and increases its viscosity and elasticity. Kansui is known to change the color of the dough through alkalization of the flavonoids in the dough (The Wheat Society of Japan, 1981). In the Yunnan Province of China, baking soda (sodium bicarbonate) is often added to Tartary buckwheat dough, when making steamed bread, to change the vivid dough color and reduce the bitter taste (Inoue, 2004). Since alkaline additives are used both for wheat and Tartary buckwheat dough for the purpose of coloring the dough yellow, the pigments that change the flour color from white to yellow through alkalization are suggested to be polyphenolic compounds such as the flavonoids.

In the present study, a significant correlation was not found between the flour or hull color and the altitude of the collection site. The content of the polyphenolic compounds that influence the color of the buckwheat flour usually varies with temperature (Luthar and Kleft, 1999) and light quality (Lee et al., 2001). If there is a significant difference among the varieties in the response of the accumulation of the polyphenolic compounds to the environmental condition, the rank of the varieties for color and antioxidant activity may vary with the cultivation site. Therefore, it is felt that G (genotype) × E (environment) field experiments are required in the future to help elucidate these responses.

In addition, precise identification of the compounds that are responsible for the varietal difference of the antioxidant activity and color of Tartary buckwheat flour is also required in future.

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The combined effects of elevated UV-B radiation and selenium on Tartary buckwheat (*Fagopyrum tataricum*) habitus

Barbara BREZNIK[1], Mateja GERM[2], Alenka GABERŠČIK[3] and Ivan KREFT[4]

[1] *Limnos Company for Applied Ecology d.o.o., Podlimbarskega 31, SI-1000 Ljubljana, Slovenia*
[2] *National Institute of Biology, Veena pot 111, SI-1000, Ljubljana, Slovenia*
[3] *Department of Biology, Biotechnical Faculty, University of Ljubljana, Veena pot 111, SI-1000 Ljubljana*
[4] *Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1001 Ljubljana, Slovenia*

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ABSTRACT

The possible ameliorative effect of selenium addition via the leaves on UV-B treated Tartary buckwheat plants (*Fagopyrum tataricum*) was monitored during a three month field experiment in the Botanical garden at Ljubljana (altitude 320 m above see level, 46°35'N, 14°55'E). The Tartary buckwheat was exposed to four treatments: ambient UV-B radiation, ambient UV-B radiation with selenium (1 mg/l), enhanced UV-B radiation (corresponding to a 17% stratospheric ozone reduction), and enhanced UV-B radiation with selenium. At the end of the experiment morphological analysis was performed. Differences in all measured parameters i.e. aboveground and underground biomass, primary branching, number of nodes, petiole length and seed production were determined. Our results showed a slight negative effect of selenium on primary branching and on seed production. UV-B treatments showed a significant effect on the Tartary buckwheat habitus. The interaction between UV-B radiation and selenium for underground biomass was elucidated.

INTRODUCTION

The ozone layer constitutes a protective atmospheric filter against biologically harmful solar UV radiation (Correia et al., 1998). During the past two decades a significant reduction in the stratospehric ozone layer, caused by contamination with the man-made chlorofluorocarbons, nitrous oxides, methyl bromide and methane has occured (Caldwell and Flint, 1994; Correia et al., 1999). The result of stratospehric ozone depletion is the enhancement of UV-B radiation at the Earth's surface.

Many investigations researching the influence of UV-B radiation on different plant species have been carried out during the last 25 years (Caldwell et al., 1998). The main conclusions of these studies was that UV-B radiation effects are species specific (Stephanou and Mantos, 1998) and depend on the balance of potential damage and the induction of protective mechanisms in the plants (Gaberščik et al., 2001). UV-B stress can promote the accumulation of active oxygen species in plants (Seppänen et al., 2003). The abnormal accumulation of these species and their derivates triggers radical chain reaction that may cause damage to such biomolecules as chlorophyll, proteins, lipids and nucleic acids and thus promote cell senescense and cell death (Xue et al., 2001). Plants with high antioxidative capacitiy are more tolerant to UV-B induced oxidative stress.

Selenium is an essential trace element for both humans and livestock (Qiuhui et al., 2002). People in areas low in selenium are living on selenium-poor food and therefore have low selenium intake (Conor, 1998). Epidemiological evidence has suggested that low Se intake may increase the risk of cardiovascular diseases and cancer (Turner et al., 1991). Furthermore, many studies have revealed its antioxidative role in humans and animals (Xue et al., 2001; Hartikainen et al., 2000) and consequently its ability to reduce the negative effects of elevated UV-B radiation on organisms. Recent findings on lettuce (*Lactuta sativa* L.) and ryegrass (*Lolium perenne* L.) suggeste that selenium can increase the tolerance of plants to UV-B induced oxidative stress, may cause a delay in senescence and promote the growth of the agening seedlings (Seppanen et al., 2003).

Changes in regional climates may require changes in agriculture. Harsher conditions may eliminate the less tolerant crops and promote others. It appears that the buckwheat species could become an important alternative crop. Buckwheat thrives at higher altitudes, which are not suitable for rice and other cereals (Gaberščik et al., 2002). It can grow without the use of chemicals when following sustainable agriculture practices that develope and extende enviromentally conscious technologies (Bonafaccia et al., 2003). Its products are known for their resistant starch and it is an important source of antioxidative substances, trace elements and dietary fibre (Bonafaccia et al., 2003). Wild Tartary buckwheat grows in southern and western China, the Tibetan plateau, and in the height Himalayan hills of Nepal, India and Pakistan (Ohnishi, 2000). The domestication processes of Tartary buckwheat has still not been elucidated (Ohnishi, 2000). Tartary buckwheat

is also used as a medical plant (Lee et al., 2001) to aid in stomach disorders (Campbell, 1997), treat choking, ulcers, haemostasis and for bathing wounds (Lee et al., 2001).

The purpose of this research was to measure the effects of increased UV-B radiation on the buckwheat species *Fagopyrum tataricum*, and to find out the possible role of selenium in mitigating its harmful effects. We examined the morpohological responses that are the major result of altered physiological process in plants induced by UV-B radiation.

MATERIAL AND METHODS

Plant material

Seeds of Tartary buckwheat (*Fagopyrum tataricum*), a domestic variety from Luxembourg, were sown in sandy soil in two pots (50×50×19 cm) per treatment in an outdoor research plot (Botanical garden, University of Ljubljana: altitude 320 m above see level, 46°35'N, 14°55'E) at the end of May. Ten plants, growing under each treatment, were removed randomly from the 100 plants that were sown in pots per treatment and were used for the growth analysis as outlined below.

Growth conditions

The UV-B supplement system was designed as previously described (Björn et al., 1993). Two different treatments were applied: simulation of 17% ozone depletion (20 cm above ground level) (UV-B) using Q-Panel UV-B 313 lamps (Cleveland, OH, USA), filtered with cellulose diacetate filters, which eliminate the UV-C range (radiation of wavelength lower than 280 nm) and ambient radiation (control system) with Q-Panel UV-B 313 (Björn and Teramura, 1993) lamps filtered with Mylar foil, which eliminates wavelengths below approximately 320 nm. The (UV-B) and control systems consisted of six Q-Panel UV-B 313 fixed in an aluminum frame (2.0×1.2 m) and was timer controlled. The doses simulating 17% ozone depletion were calculated and adjusted weekly according to the program developed by Björn and Murphy (1985) using the generalized plant action spectrum (Caldwell, 1968). Ambient UV-B, UV-A and PAR were monitored by a three-channel dosimeter (ELDONET) belonging to the European Light Dosimeter Network.

The Tartary buckwheat was exposed to four treatments: control (ambient UV-B radiation), control+Se (ambient UV-B radiation with selenium in the form of Na selenate (1 mg/l)), UV-B (enhanced UV-B radiation corresponding to 17% stratospheric ozone reduction) and UV-B+Se (enhanced UV-B radiation with selenium).

Growth analysis

Plant biomass: The sample plants (ten per treatment) were dried at ambient temperature until they reached a constant weight (10 days). After drying aboveground and underground parts of the samples were weighed separately.

Branching, number of nodes and seeds, and petiole length: The number of branches, nodes and seeds per each plant and petiole length were recorded at the end of experiment before the samples were dried.

Statistical analysis

The sample plants used for the analysis were randomly chosen from 100 plants grown under each treatment. The significance of the effects of UV-B radiation, selenium addition, and the interaction of both parameters was tested by one-way ANOVA and multifactor ANOVA (Statgraphics Version 4).

RESULTS AND DISCUSSION

Aboveground and underground biomass

Tartary buckwheat expressed a reduced aboveground and underground biomass under enhanced UV-B radiation in comparison to ambient UV-B radiation. UV-B induced reduction in both aboveground and undeground biomass had also reported from results of Gaberščik et al. (2002) for common buckwheat. They reported approximately a 24% dry weight reduction of the shoots and a 14% reduction of the roots of common buckwheat. The sigificant differences (according to one-way ANOVA) in shoot dry weight for Tartary buckwheat in the present study was observed between the control and UV-B+Se treatments (Fig. 1). Two-factors ANOVA also revealed a significant difference between the control and elevated UV-B treatment (Table 1). UV-B radiation has also been reported to have a negative effect on the production of the aboveground biomass for various bush bean cultivars (Saile-Mark et al., 1997) and Portuguese Barbela wheat

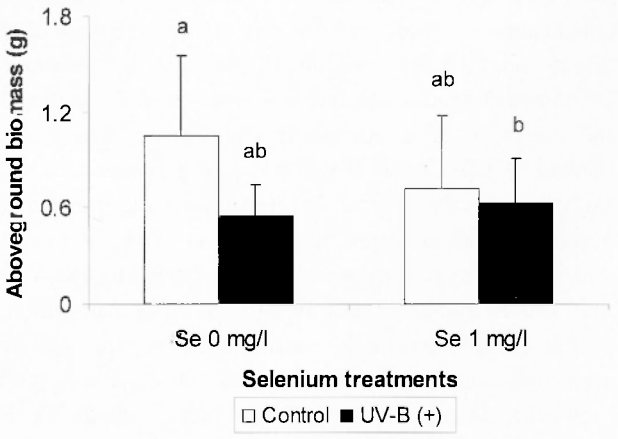

Fig. 1. Aboveground biomass for Tartary buckwheat (*Fagopyrum tataricum*) under four different treatments. Columns not sharing the same letter are significantly different (one-way ANOVA; 95% LSD method).

Table 1. Results (p values) of multifactor ANOVA for aboveground biomass, underground biomass, primary branching, number of nodes, petiole length and number of seeds of Tartary buckwheat

	Abovegr. biomass	Undergr. biomass	Primary branching	No. of nodes	Petiole length	No. of seeds
Main effects						
UV-B	0.0157*	0.0214*	0.0011**	0.0153*	0.0250*	0.0090**
Se	0.4342	0.3654	0.0764	0.3067	0.3100	0.0685
Interaction						
UV-B x Se	0.8374	0.0072**	0.7888	0.2349	0.2430	0.2877

*, **: Significant at 0.05 and 0.01 level, respectively.

(Correia et al., 1999). In the present study the underground biomass reflected patterns similar to aboveground biomass. We observed a significant reduction in the underground biomass between the control and the control+Se, the control and UV-B, and the control and UV-B+Se treatments (one-way ANOVA) (Fig. 2). The significant interaction between the UV-B and selenium treatments was observed only for the underground biomass (Table 1). On the contrary, Valkama et al. (2003) did not observe any interaction between UV and selenium for root biomass of strawberry. However, in the present study it appeared that selenium had a negative effect on the underground biomass of Tartary buckwheat under ambient growing conditions (Fig. 2). Despite a significant interaction between UV-B radiation and selenium for the production of underground biomass we did not notice any significant differences between the treatments with or without selenium. The same results were observed for production of the aboveground biomass (Table 1).

The reduction of the buckwheat dry weight may have been a consequence of UV-B induced changes in morphogenetic and physiological processes (Correia et al., 1999), i.e. reduced enzyme activities (Saile-Mark, 1997), lower efficiency of the photosystem II and stomatal conductance (Saile-Mark et al., 1997), and a disturbance in water economy and water deficiency (Larcher, 1995). The reduced biomass, might also be a consequence of carbohydrate partitioning (Gaberščik et al., 2002). A decrease in leaf area and lower tillering contribute to lower biomass accumulation in UV-B treated plants has previously been reported (Correia et al., 1997), as was also revealed from the present study (Fig. 3).

Our results also supported Hartikainen findings (Hartikainen et al., 2000) regarding the pro-oxidant and anti-oxidant role of selenium in plants. Light stress can promote the accumulation of active oxygen species (AOS) in the chloroplast in situations where the anti-oxidant capacity to detoxify AOS is exceeded (Seppänen et al., 2003). Selenium promotes scavenging of H_2O_2 produced through increased glutathione peroxidase (GSH-Px) (Hartikainen et al., 2000). Simultaneously with the enhanced GSH-Px there is enhanced spontaneous dis-

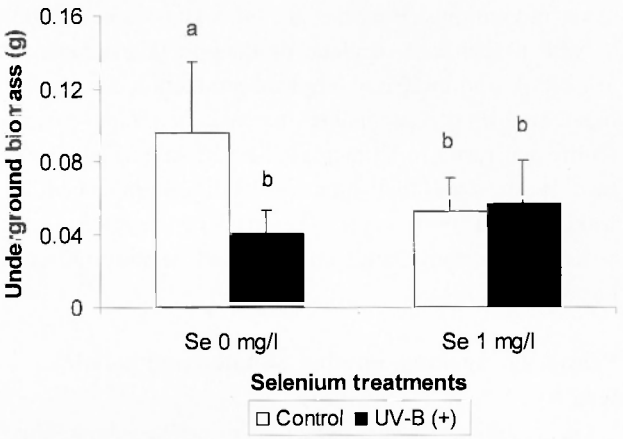

Fig. 2. Underground biomass for Tartary buckwheat (*Fagopyrum tataricum*) under four different treatments. Columns not sharing the same letter are significantly different (one-way ANOVA; 95% LSD method).

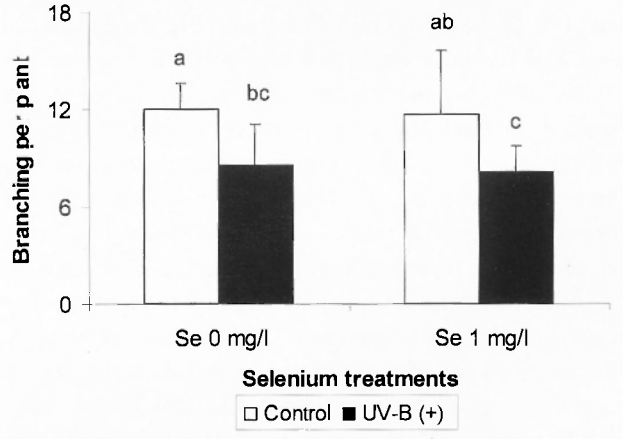

Fig. 3. Branching per plant for Tartary buckwheat (*Fagopyrum tataricum*) under four different treatments. Columns not sharing the same letter are significantly different (one-way ANOVA; 95% LSD method).

proportion of superoxide radicals and, consequently, a reduced need for the scavenger, superoxide dismutase (SOD) (Hartikainen et al., 2000). Furthermore, at non-toxic selenium levels, the decrease in superoxide radicals through spontaneous disproportion diminished the production of lipid peroxide radicals (LOO) (Xue et al., 2001). In this manner plants eliminate AOS and protect

the chloroplast from damage caused by AOS. The growth-stimulating effect of selenium is therefore related to selenium's antioxidative function (Xue et al., 2001). In contrast to the positive role of selenium, some studies have revealed that selenium may have toxic properties, especially at higher doses. In lettuce, ryegrass and potato the growth and dry weight decreased under high doses of selenium (1 mg/kg dry weight) (Xue et al., 2001; Hartikainen et al., 2000; Seppänen et al., 2003). The toxicity of selenium to plants has generally been ascribed to altered physiological processes. Selenium can indiscriminantly substitute for sulphur and incorporate Se-amino acids into proteins (Brown et al., 1982; Hartikainen, 2000) as well as enhance ethylene production (Hartikainen et al., 2000). The increased ethylene production can modify membrane lipid components, increase membrane permeability and result in an increased K^+ leakage. Thus it can be hypothesized that high Se addition enhances K^+ leakeage and more water is retained in the intercellular spaces to counterbalance an increased osmotic pressure (Xue et al., 2001).

Primary branching, number of nodes and petiole lenght

Our results showed a reduction in primary branching, number of nodes and petiole length under elevated UV-B radiation in comparison to ambient UV-B radiation. According to the one-way ANOVA a significant reduction in the parameters were obtained between the control and UV-B, the control and UV-B+Se, the control+Se and UV-B+Se for primary branching (Fig. 3), the control and UV-B, the control and UV-B+Se for number of nodes (Fig. 4), the control and UV-B+Se, and the control+Se and UV-B+Se for petiole length (Fig. 5). Most researchers have reported increased branching under enhanced UV-B which was in contrast to the present results. The studies on *Vicia faba* by Mejikamp et al. (2001) revealed increased tillering. Deckmyn et al. (1999) reported similar results for *Lolium* sp. The response of plants to enhanced UV-B levels was possibly the consequence of the loss of apical dominance, which could be caused by a reduction in indol-3-acetic acid (IAA) activity (Mejikamp et al., 2001). It is suggested that IAA can be destroyed directly by UV-B or that IAA activity can be reduced by an interaction with the quercetin flavonoids (Mejikamp et al., 2001).

Reproduction

Succesful reproduction is an important aspect for agricultural plants. The most sensitive time for developing plants is the transition from the vegetative to the reproductive phase (Teramura et al., 1987). Poorly protected reproductive organs may be susceptible to the damaging effects. The significant differences in seed production

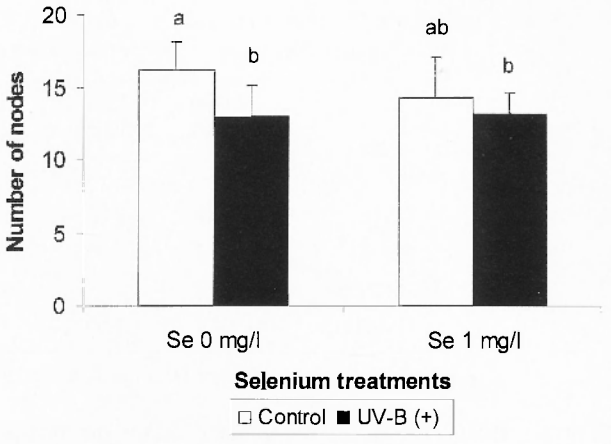

Fig. 4. Number of nodes for Tartary buckwheat (*Fagopyrum tataricum*) under four different treatments. Columns not sharing the same letter are significantly different (one-way ANOVA; 95% LSD method).

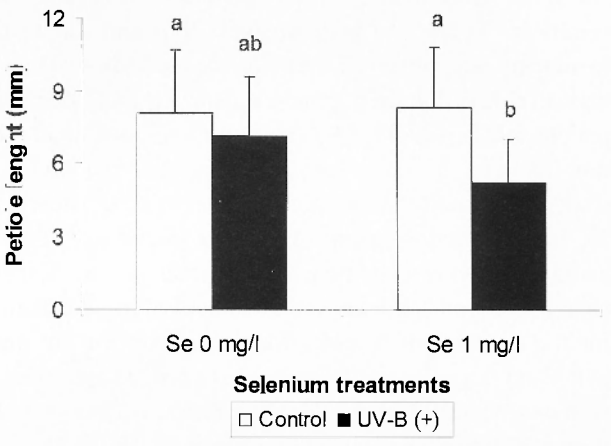

Fig. 5. Petiole length for Tartary buckwheat (*Fagopyrum tataricum*) under four different treatments. The significant differences between treatments is indicated by letters a and b (one-way ANOVA; 95% LSD method).

between the UV-B treatments were observed and we also revealed the possibility of selenium influence on Tartary buckwheat reproduction (multifactor ANOVA) (Table 1). According to the one-way ANOVA, significant differences were obtained between the control and UV-B+Se, the control+Se and UV-B+Se treatments (Fig. 6). Common buckwheat was reported to show very similar responses to enhanced UV-B levels with the seed production for reduced, ambient and enhanced UV-B being 33, 19 and 13 seeds per plant (Gaberščik et al., 2002). Al-Oudat et al. (1998) also observed a reduced number of seeds in *Vicia faba*. Scientists ascribe lower seed production to a shift in the time of flowering, which can result in insufficient insect pollinators available at the time of flowering (Al-Oudat et al., 1998) or to lower pollen quality which could be directly affected by UV-B radiation (Demchik et al., 1996). No interaction between UV-B and selenium treatments (multifactor ANOVA) (Table 1) was observed in the present study.

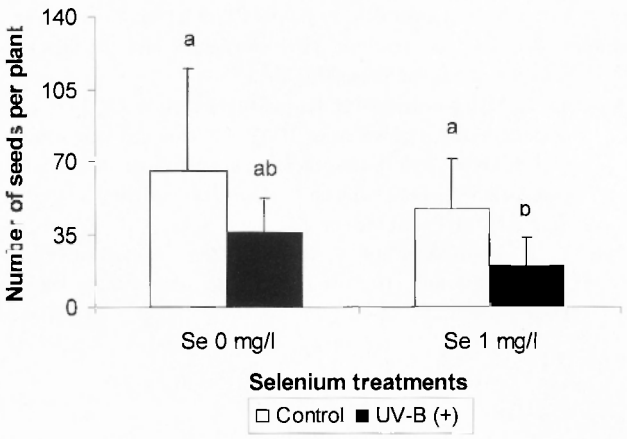

Fig. 6. Number of seeds per plant for Tartary buckwheat (*Fagopyrum tataricum*) under four different treatments. Columns not sharing the same letter are significantly different (one-way ANOVA; 95% LSD method).

CONCLUSION

This study demonstrated responses of Tartary buckwheat to enhanced UV-B radiation, selenium and combined UV-B and selenium treatments. Enhanced UV-B treatment affected the aboveground and underground biomass, tillering, number of nodes, petiole length and reproduction. The addition of selenium induced a slightly negative effect on primary branching and seed production, while it did not express any clear effect on the other parameters. We also observed an interaction between UV-B radiation and selenium for underground biomass. Tartary buckwheat was revealed to be an agricultural plant which was quite sensitive to elevated UV-B radiation.

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Fagopyrum 21: 65–69 (2004)

Effects of environmental factors on the chemical characteristics of common buckwheat in relation to flour texture
1. Variation in amylose and crude protein content of seeds collected at various sites in Japan

Naoto INOUE, Kaori FUJITA, Zhongfa YANG, Masakazu KATO, Shu-hei UEHARA, Motoyuki HAGIWARA and Akio UJIHARA

Faculty of Agriculture, Shinshu University, Minamiminowa, Nagano 399-4598, Japan

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ABSTRACT

The content of amylose and crude protein in the flour from seeds of a tetraploid buckwheat variety which was grown on 110 different cultivation sites were examined. The flour samples were processed so as to produce a uniform milling percentage as possible. The average, standard deviation, and maximum and minimum values of the amylose content were 18.7, 1.8, 23.5 and 14.9%, respectively. There was a significant negative correlation between the content of amylose and crude protein ($r=-0.754$, $P<0.001$). The geographical variation in the content of amylose and crude protein were examined: (1) the amylose content in the flour was higher in the western region of Japan than in the eastern part, (2) the crude protein content was lower in the western than in the eastern part in Japan, and (3) the higher the altitude of the cultivation sites the lower the amylase content ($r=-0.450$, $P<0.001$).

INTRODUCTION

Common buckwheat flour which is manufactured by the millers is consumed as 'soba' noodles in Japan and most of the flour is sold by the millers directly to 'soba' restaurants (Suzuki, 2003). Therefore, the millers are sensitive to the consumers' demands for flour quality and respond to these demands. The viscosity of the buckwheat flour is one of the most important physical traits in the production of buckwheat noodles in Japan. Buckwheat millers have a large amount of traditional knowledge which indicates that buckwheat cultivated in the cool regions of the eastern parts of Japan, Hokkaido and Tohoku, bear seeds that produce a soft flour (higher quality) and that buckwheat cultivated in the warm regions in the south western part of Japan, Kyushu, Shikoku and Chugoku bear seeds that produce a hard flour (Takou, 1975). However, the reason for this relationship is not understood, especially in relation to the chemical traits of flour.

Furusawa and Kobayashi (1963) and Furusawa and Miyashita (1964, 1965) reported that the content of amylose, protein and other chemical constituents that are related to flour viscosity, varied with the cultivation site and genotype. Recently, Ikeda et al. (1999) pointed out that the crude protein content in common buckwheat flour is an important factor responsible for its textural characteristics. From these reports, we hypothesized that the environmental factors during the growing period of buckwheat not only influence its flour protein content which determines flour viscosity, and which buckwheat millers express as hard or soft, but also may influence the amylose content. However, there is no report on the geographical variation of the content of amylose and protein in buckwheat flour in Japan and the relationship between the content of the two chemical components in buckwheat flour.

In this report, common buckwheat seeds that were harvested at various cultivation sites in Japan were analyzed for variation of amylose and crude protein content in the flour, which may influence the flour texture. The relationship between the content of amylose and crude protein in the flour was also analyzed.

MATERIALS AND METHODS

Materials

A tetraploid variety "Shinshu-oosoba" of common buckwheat (*Fagopyrum esculentum* Moench), which was developed by Dr. Ujihara and TAKANO Co. Ltd., was used in the present experiments to reduce the effect of genotypic difference on the chemical properties of the flour. This variety has low heterozygosity as any outcrossing with diploid material results in sterile triploid progeny which do not add heterozygosity to the population. Seed samples from a field grown at Shinshu University in 1985 were supplied to designated farmers in all prefectures of Japan except Okinawa in 1986 and each farmer was requested to cultivate "Shinshu-oosoba". The seeds were sown in the summer period at each site and

fertilizer application, plant density and other management practices followed local and traditional methods at each site. The seeds were stored at 10°C in a cold store room with a dehumidifier before milling. The seeds from the crops which were harvested at the 110 sites, as shown in Table 1, were processed to analyze the chemical traits.

Milling and chemical analysis of flour

Sixty seeds from each cultivation site were ground in a mortar and passed through a 100 mesh sieve (pore size 0.16 mm) to provide uniformity of the flour particles and to eliminate the hull, just before chemical analysis in 2002. The milling procedure was adjusted to make a uniform ratio of the flour weight before sieving to seed weight before grinding (milling percentage).

One hundred mg of air-dried samples were added to 10 ml of 0.5 N NaOH solution and the final volume adjusted to 100 ml with distilled water. The solution was left standing overnight at room temperature, and the amylose content of the solution were then determined by an Autoanalyzer-II (BRAN+LUEBBE, Germany). 60 samples were selected for protein analyses based on their amylose content, so that the 60 samples covered the entire distribution of amylose content evenly. The content of total nitrogen (%) was determined for these 60 samples by a C-N Corder (YANAGIMOTO, Japan). The crude protein content was calculated as total nitrogen×6.25. In the present report, all of the chemical values are expressed on an air-dried weight basis.

Table 1. Materials used for chemical analysis

Collection site		Number of Sample
District (locality[1])	Prefecture	
Tohoku (N)	Iwate	3
	Akita	3
	Yamagata	2
	Miyagi	2
	Fukushima	5
Kanto (C)	Kanagawa	2
	Chiba	1
	Ibaraki	4
	Saitama	2
	Tochigi	3
	Gunma	4
	Yamanashi	3
Hokushinetsu (C)	Nagano	45
	Niigata	4
	Ishikawa	1
Thokai (C)	Gifu	3
	Shizuoka	2
Kinki (W)	Hyogo	1
	Kyoto	1
Chugoku (W)	Tottori	1
	Shimane	2
	Okayama	1
	Hiroshima	2
	Yamaguchi	3
Shikoku (SW)	Ehime	3
Kyushu (SW)	Fukuoka	2
	Nagasaki	2
	Kumamoto	3
Total		110

[1] N, C, W and SW denote the northern, central, western and south western parts of Japan, respectively.
Refer to Fig. 4.

RESULTS

Fig. 1 shows the frequency distribution of the amylose content in the flour from samples collected at the various sites. The mean, standard deviation, maximum and minimum values of amylose content were 18.7, 1.8, 23.5 and 14.9%, respectively. For crude protein, the mean, standard deviation, maximum and minimum values were 9.8, 1.9, 13.4 and 6.4%, respectively.

In this experiment, the mean and standard deviation of milling percentage were 57.0 and 4.0%. No correlation between the amylose content and the milling percentage was found (Fig. 2). There was a significant negative correlation ($r=-0.754$, $P<0.001$) between the amylose and crude protein content in the samples as shown in Fig. 3.

The values of the amylose content in the 60 samples which were randomly selected were then plotted on a map of Japan (Fig. 4). The amylose content of flour tended to be higher in the western part of Japan than in

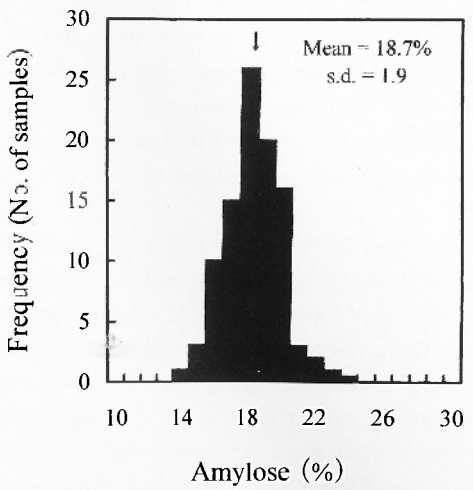

Fig. 1. Frequency distribution of amylose content in buckwheat flour.

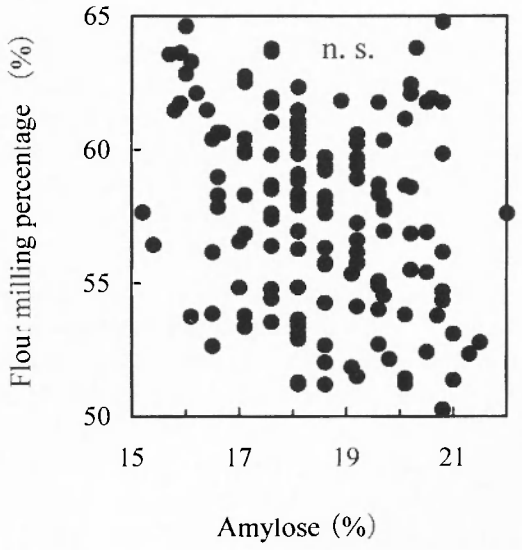

Fig. 2. Relationship between flour milling percentage and amylose content in buckwheat flour.

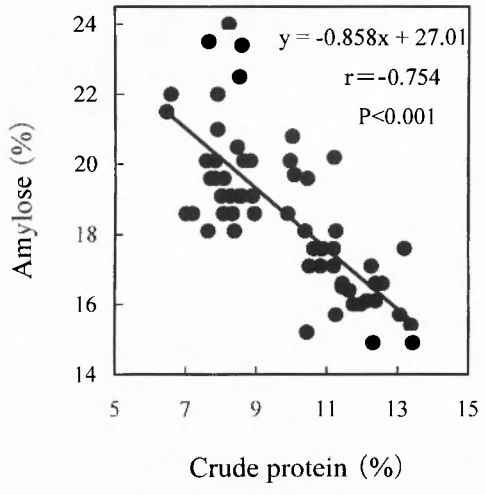

Fig. 3. Relationship between amylose and crude protein contents in buckwheat flour.

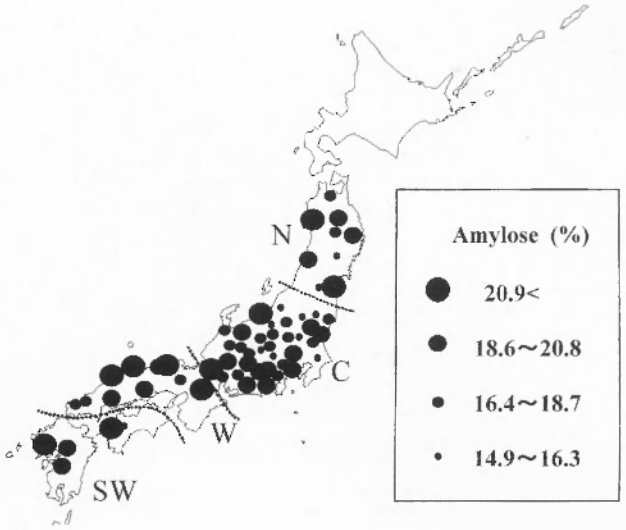

Fig. 4. Geographical variation in the amylose content of buckwheat flour.
Signs in the figure denote the locality, see Table 1.

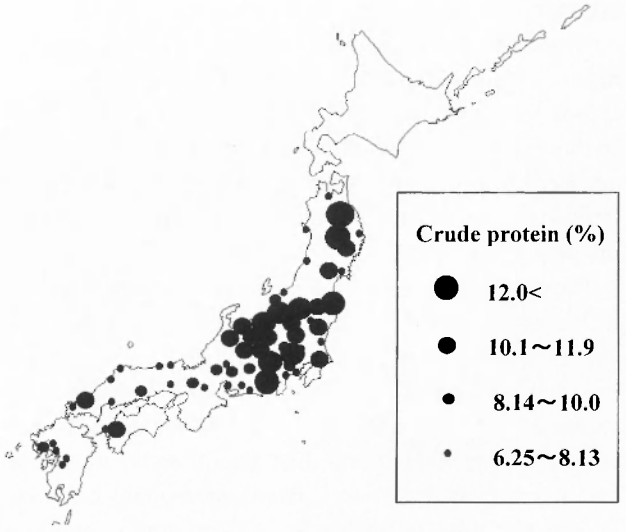

Fig. 5. Geographical variation of crude protein content of buckwheat flour.

the eastern part and in the costal regions than in the inland regions. However, it was found to vary greatly between sites, even in the same district (region).

On the other hand, the crude protein content was clearly lower in the western part than in the eastern part of Japan (Fig. 5).

The amylose content of the flour of all the samples which were collected were plotted against the altitudes of cultivation sites and are shown in Fig. 6. The amylose content was found to be significantly decreased with an increase in altitude of the cultivation sites ($r=-0.450$, $P<0.001$).

DISCUSSION

Flour texture, as evaluated with a texturometer, as well as the crude protein content of the buckwheat flour may have drastic changes depending on the particle size of flour (Ikeda et al., 1999). This suggests that the milling percentage considerably influences the chemical constituents and physical property of buckwheat flour. Therefore, we made an effort to maintain a uniform as possible milling percentage in this experiment. Although the milling percentage may have varied slightly, there was no correlation found between the milling percentage and amylose content of flour samples. This indicates that the variation in amylose content of the buckwheat flour which was observed in this study was not caused by any difference in the milling process but by environmental factors at cultivation sites.

The buckwheat variety used in this study was tetraploid. Therefore any pollination from diploid plants in adjacent fields would result in sterile triploid plants. It is

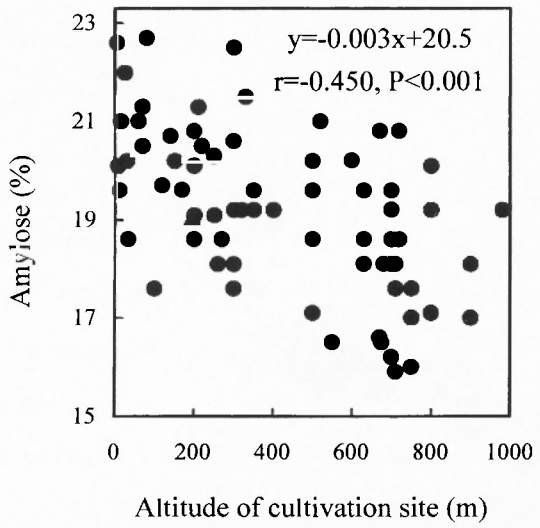

Fig. 6. Relationship between the amylose content of buckwheat flour and the altitude of cultivation site.

therefore considered to have a lower heterozygosity when compared to diploid varieties which can have increased heterozygosity due to outcrossing. Therefore, the variation in the chemical traits of the flour samples observed in this study would be minimized by genetic factors and appeared to be caused by the difference in the meteorological conditions and/or cultivation management during the growing period.

The range of variation in the amylose content observed in this study was 8.6%, and was smaller than the 14% (Min., 14.2%; Max., 28.2; mean, 22.7%) previously reported by Inoue and Nakata (2001). However, they examined 250 common buckwheat varieties which were collected from Eurasia and then determined the amylose and starch content. When it is considered that only one variety was used in this study, the variation in amylose content appeared to be quite high.

In this study, a wide variation was also observed in the content of crude protein as well as in the amylose content among the flour samples, and a significant negative correlation was observed between them (Fig. 3). There has been no report on a negative relationship between these constituents, and therefore the present results appear to be significant in studies on the chemical and physical components of buckwheat. Ikeda et al. (1999) compared the textural characteristics of common buckwheat flour samples having different particle sizes with their protein content and found that the protein content in the flour was an important factor in determining flour textural characteristics. There are many reports which show that the stickiness of rice increases as the content of amylose decreases (Chikubu, 1995; Asaoka et al., 1985; Matsue et al., 2002). In common buckwheat, however, only limited information is available on the correlation of textural characteristics with amylose and crude protein content of the flour. The present study showed that the content of amylose and crude protein of common buckwheat flour were influenced by the environmental conditions at the cultivation site. Net assimilation may increase the starch and also the amylose content in seed dry matter and may coincidentally decrease the content of protein which is abundantly present in the germinal layer and in the hypocotyl.

Ikeda et al. (1999) revealed that crude protein content was negatively correlated with textural characteristics of common buckwheat flour, i.e., hardness, cohesiveness, springiness, and chewiness, but not with adhesiveness of heated-dough made from buckwheat flour. In our experiment, the amylose content was found to be correlated negatively with crude protein. This suggests that the amylose content may correlate positively with textural characteristics, i.e., hardness in common buckwheat flour.

The 'soba' noodle makers in Japan, called Sobaya in Japanese, consider that the locality of buckwheat production is the most important factor when choosing the best flour which can be easily extended and does not break during boiling. Takou (1975) pointed out that, based on his own experience, a difference in hardness between common buckwheat flour in the western (corresponding to W, SW and the western part of C in Fig. 4) and eastern Japan (corresponding to N and the eastern part of C in Fig. 4). He also suggested that the variation in hardness of the flour produced in the same region probably was probably due to different elevations (altitude). Such empirical knowledge of the millers correlates well with the results of our experiment; that flour produced in the eastern part of Japan or in the high altitude regions is characterized by relatively low amylose and relatively high protein content.

Iino and Inoue (2000) investigated historical data from the Edo period to 1875, and reported that Kawakami village in Shinshu (the old name of Nagano prefecture) which is located in a high mountain area in central Japan, and is situated between the two major consuming areas, Edo (the old name of Tokyo) and Osaka, had became a reputed production center of (soft) common buckwheat flour since the 1800s, the late Edo era. The softness of the flour produced in such a highland area was suitable for buckwheat noodle processing, as the dough is easily extended, cut and boiled without breaking.

The results of this study strongly suggest that environmental conditions during the growing season of common buckwheat cause a wide variation in both amylose and crude protein contents of its flour. However, the mechanism of how the environmental factors influence these chemical characteristics of common buckwheat is not yet clarified. The relationship between the meteorological factors during the growing period of common buckwheat and the chemical characteristics of its flour that deter-

mines the textural characteristics of the flour will be evaluated in the future.

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Fagopyrum 21: 71–7[illegible] (2004)

Characterization of buckwheat dough and noodles with respect to mechanical characteristics

Kiyokazu IKEDA*[1], Yuya ASAMI[2], Rufa LIN[3], Ivan KREFT[4], Rie ARAI[1] and Kyoden YASUMOTO[5]

[1] *Faculty of Nutrition, Kobe Gakuin University, Nishi-ku, Kobe 651-2180, Japan*
[2] *Kyoto College of Nutritional & Medical Sciences, Ukyo-ku, Kyoto 616-8376, Japan*
[3] *Shanxi Academy of Agricultural Sciences, Taiyuan, Shanxi, Province, China*
[4] *Biotechnical Faculty, University of Ljubljana, 1001 Ljubljana, Slovenia*
[5] *Department of Food and Nutrition, School of Living Studies, Sugiyama Jogakuen University, Chikusa-ku, Nagoya 464-8662, Japan*

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Key words: mechanical characteristics, palatability

ABSTRACT

The present study was conducted to characterize buckwheat foods by multivariate analyses, i.e., principal component analysis and cluster analysis, with respect to their mechanical characteristics. Cluster analysis with respect to textural characteristics was shown to classify buckwheat dough made from various flours and cultivars into several groups. Principal component analysis with respect to product rupture and tensile analyses was shown to classify various buckwheat noodles into different groups. We concluded that multivariate analyses with respect to mechanical characteristics can demonstrate preference mapping of buckwheat foods in view of their palatability and acceptability.

INTRODUCTION

Buckwheat (*Fagopyrum* spp.) is an important crop in some regions of the world (Kreft et al., 2003; Ikeda, 2002). Buckwheat flour contains essential nutrients such as protein (Ikeda et al., 1991) and minerals (Ikeda and Yamashita, 1994) at high levels. Thus, buckwheat contributes as an important dietary source of such essential nutrients.

There is a large variety of buckwheat foods globally. Noodles made from buckwheat flour-water dough are popular in some countries such as Japan, Korea and China. In Japan, a large variety of buckwheat noodles are available, e.g., dried noodles (kan-men), precooked noodles (yude-men) and hand-made noodles (te-uti-men). Furthermore, there is a large variety of buckwheat noodles with various types of dough-binders, e.g., wheat flour, egg, seaweed, yam flour etc. In view of their cooking and processing characteristics, a large amount of attention has been paid to the palatability and acceptability of buckwheat foods including noodles. However, there are still many unanswered questions on the palatability and acceptability of buckwheat foods. Although many buckwheat noodles that are available may be palatable and acceptable respectively, the determinants that are responsible for their palatability and acceptability remain unclear. In view of such a background, clarifying the palatability and acceptability of buckwheat is a subject of great interest. The mechanical characteristics of buckwheat foods may be an important quality attribute affecting their palatability and acceptability. Hence, characterization of the mechanical features of buckwheat foods is an important subject.

On the other hand, there are two different species of buckwheat which are cultivated, i.e., common buckwheat (*F. esculentum* Moench) and Tartary buckwheat (*F. tataricum* Gaertner). The possibility that Tartary buckwheat may exhibit some beneficial effects on human health has been suggested (Lin et al., 1998), although the exact mechanisms involved remain unclear. In Japan, various products, including noodles, made from Tartary buckwheat are currently becoming available. Therefore, characterization of Tartary buckwheat is an important subject from the view of food processing.

The present study was conducted to characterize foods made from buckwheat flour, including common and Tartary buckwheat flours, with respect to their palatability and acceptability, especially as it is related to their mechanical characteristics.

MATERIALS AND METHODS

Materials

The buckwheat samples analyzed in this study consisted of twenty-eight different kinds of buckwheat; five cultivars of common buckwheat, four cultivars of Tartary buckwheat (*F. tataricum* Gaertner), three different kinds of commercially-prepared buckwheat flour, thirteen kinds of commercially-prepared dried buckwheat noodles (kan-men in Japanese), three types of commercially-prepared

* Corresponding author

precooked buckwheat noodles (yude-men in Japanese). Each buckwheat grain sample was milled with a roller mill (Quadrumat Junior, Model No. 279002, Brabender OHG Duisburg, Germany) fitted with 231 μm-sieve, respectively. All the ground samples examined contained none or only a very small amounts of husk.

Five varieties of common buckwheat, i.e., var. Kitawase-soba, var. Hashikami-wase, var. Shinshu-oosoba (tetraploid), var. Hitati-akisoba and one local variety were subjected for texture analysis.

The Tartary buckwheat samples consisted of one Japanese and three Chinese varieties. The Japanese one, i.e., var. Hokkei No.1 was obtained from the Department of Crop Breeding, Hokkaido National Agriculture Experiment Station Research (Hokkaido, Japan). The three Chinese Tartary buckwheat samples were supplied by one of the authors (R. L.); the first sample was var. Hei Ku Qian; the second, var. Hui Ku Qiao; and the third one, var. Shoungang Bendi Ku Qiao, which was supplied as flour sample. Each Tartary buckwheat grain sample was milled with the roller mill (Quadrumat Junior, Model No. 279002, Brabender OHG Duisburg, Germany) fitted with 231 μm-sieve, respectively. The Tartary buckwheat flour obtained was subjected to mechanical analysis.

Three kinds of commercially-prepared buckwheat flour, i.e., whole buckwheat flour and flour from the outer-layer, prepared from var. Kita-wase-soba, and commercial inner-layer flour were obtained and subjected to mechanical measurements.

Thirteen kinds of commercially-prepared dried buckwheat noodles (kan-men in Japanese), and three types of commercial precooked buckwheat noodles (yude-men in Japanese) were obtained locally and were analyzed immediately after being obtained.

Analysis of protein

The total protein content (TPC) (N X 6.25) of buckwheat flours was analyzed by a Kjeltec Auto 1035 nitrogen analyzer (Perstrop Analytical Tecator, Sweden). The TPC of commercially-prepared buckwheat whole buckwheat flour was 12.3 g/100 g dry matter (DM); the TPC of commercially-prepared outer-layer flour, 15.0/100 g DM; and the TPC of commercially-prepared inner-layer flour, 4.0 g/100 g.

Mechanical measurements

Mechanical characteristics were evaluated by three different mechanical analyses in the present study: texture analysis with heated buckwheat dough; breaking analysis with buckwheat noodles; and tensile analysis with buckwheat noodles. Texture analysis of the buckwheat dough was performed with a Rheolometer RX-1600 (Iio Denki Co., Japan) according to a procedure previously described (Ikeda et al., 1997 and 1999). Breaking analysis with buckwheat noodles was performed with a Rheoner RE-3305 by a procedure previously described (Ikeda and Asami, 2000). Tensile analysis of the buckwheat noodles performed with a RT-3005D by a procedure described previously (Ikeda and Asami, 2000). The mechanical measurements were repeated five to ten times, each time with a different sample of buckwheat dough.

Statistical analysis

Statistical analyses were conducted for principal component analysis and cluster analysis using a personal computer with the program Excel (Microsoft Co., USA).

RESULTS AND DISCUSSION

Classification of buckwheat foods with respect to texture analysis

Figure 1 shows the textural characteristics of four doughs made from the inner-layer flour, outer-layer flour, whole buckwheat flour of common buckwheat, and wheat flour, respectively. There was a variation in the texture characteristics, i.e., hardness, cohesiveness, springiness and chewiness, among the four doughs (Fig. 1). Figure 2 and Table 1 shows the results of principal component analysis based on the textural values presented in Fig. 1. Principal component analysis with respect to texture characteristics can separate the four doughs into different positions on the coordinates (Fig. 2). In this connection, there are various kinds of buckwheat noodles in Japan. There are two major buckwheat noodles in Japan: one is noodles (called sarashina-soba) made from inner-layer flour (sarashina-flour); and the other is noodles (called inaka-soba (inaka means a rural region) made from whole buckwheat flour. Both types of buckwheat noodles are widely utilized in Japan. There is a difference in preference for both types of noodles in view of their palatability and acceptability: i.e., there are many Japanese people who prefer to sarashina-soba, whereas there are many Japanese people who prefer to inaka-soba. Therefore, Fig. 2 suggests a possibility that multivariate analysis such as principal component analysis and cluster analysis can demonstrate preference mapping for buckwheat foods, especially buckwheat doughs made from various buckwheat flours, in view of their palatability and acceptability. Figure 3 presents the results of the cluster analysis based on the observed textual values (direct analytical data are not shown) of doughs made from five common buckwheat and one Tartary buckwheat cultivars. This shows that cluster analysis, with respect to texture characteristics, can clearly divide doughs made from the six buckwheat cultivars. Cluster analysis can roughly divide common and Tartary buckwheat into two main groups. In a previous paper (Ikeda et al., 2003), we have shown a striking difference in the mechanical characteristics

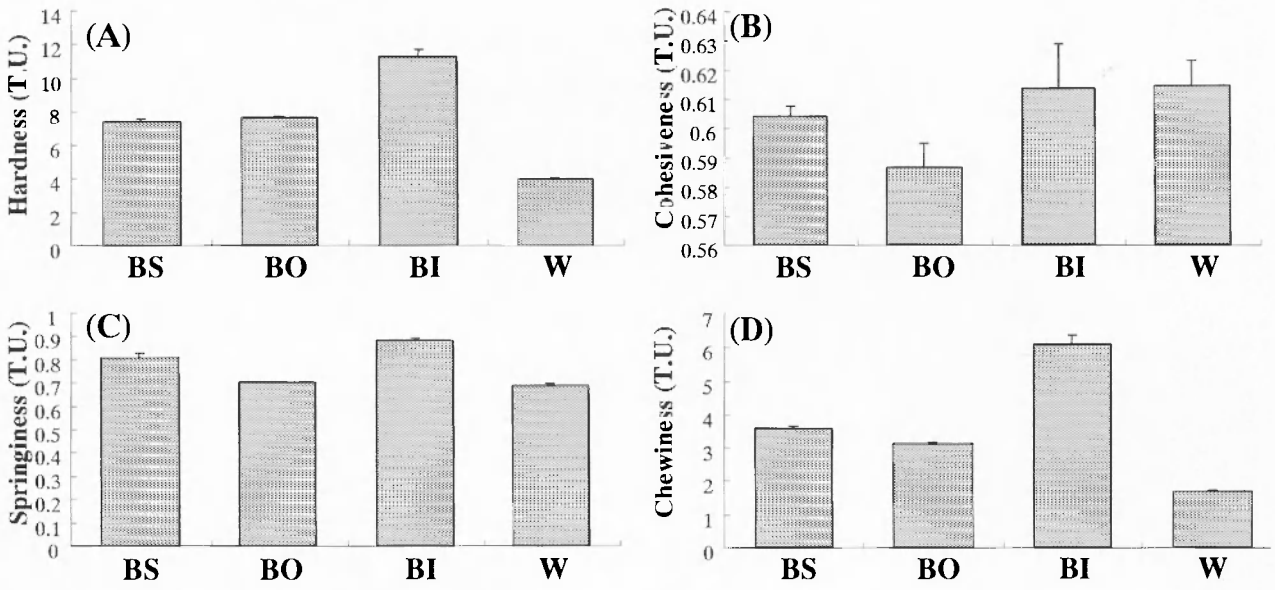

Fig. 1. Textural characteristics of various buckwheat doughs and wheat dough. Dough made from whole buckwheat whole (BS), buckwheat outer-layer flour (BO), buckwheat inner-layer flour (BI) and wheat flour (W) were analyzed.

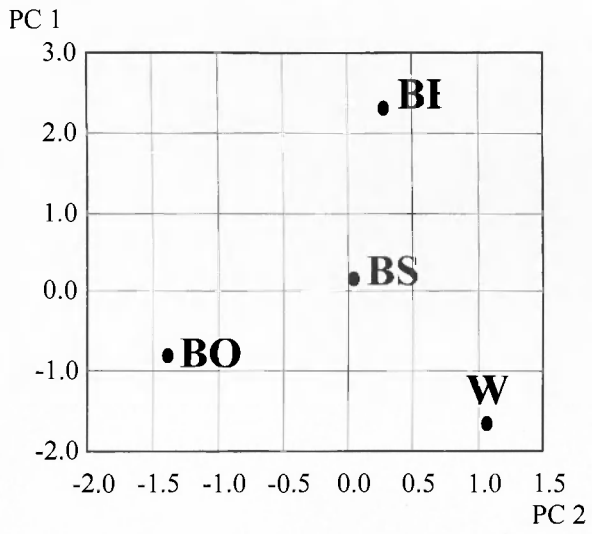

Fig. 2. Principal component analysis of the dough made from various buckwheat flours and wheat flour with respect to textural characteristics observed in Fig. 1. The abbreviations for the samples were the same as in Fig. 1.

Table 1. Eigen vector, factor loading, eigen value and contribution extracted from principal component analysis of four characters for four samples

Character	Eigen vector		Factor loading	
	PC1	PC2	PC1	PC2
Hardness	0.5565	−0.2986	0.9477	−0.3054
Cohesiveness	0.1630	0.9368	0.2775	0.9584
Springiness	0.5707	0.1411	0.9718	0.1444
Chewiness	0.5814	−0.1153	0.9901	−0.1180
Eigen value			2.90	1.05
Cumulative contribution (%)			72.50	98.66

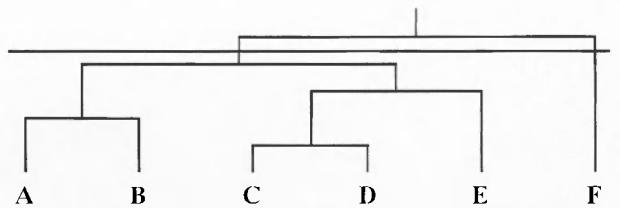

Fig. 3. Cluster analysis of dough made from various buckwheat cultivars. A, var. Kitawase-soba; B, var. Hashikami-wase; C, var. Sinshu-oosoba; D, var. Hitati-aki-soba; E, local var.; and F, Tartary buckwheat (Hokkei No. 1). Cluster analysis was performed by separation them into two major group as indicated by the horizontal bar in the figure.

between common and Tartary buckwheat. The present finding (Fig. 3) agrees well with our previous findings (Ikeda et al., 2003). Thus our findings (Figs. 2 and 3) suggest that multivariate analysis such as principal component analysis and cluster analysis can demonstrate preference mapping for buckwheat foods, consisting of doughs made from various buckwheat flours, in view of their palatability and acceptability.

Classification of buckwheat foods with respect to breaking and tensile analyses

Figure 4 shows the breaking and tensile analyses of noodles made from the inner-layer flour, outer-layer flour, whole flour of common buckwheat, and wheat flour respectively. Figure 4 shows a clear variation in the breaking and tensile analyses of the four noodles. Figure 5 and Table 2 presents principal component analysis of the breaking and tensile characteristics, observed in Fig. 4, of the four noodles made from the inner-layer flour, outer-layer flour, whole flour of buckwheat, and wheat flour. Principal component analysis shows that breaking

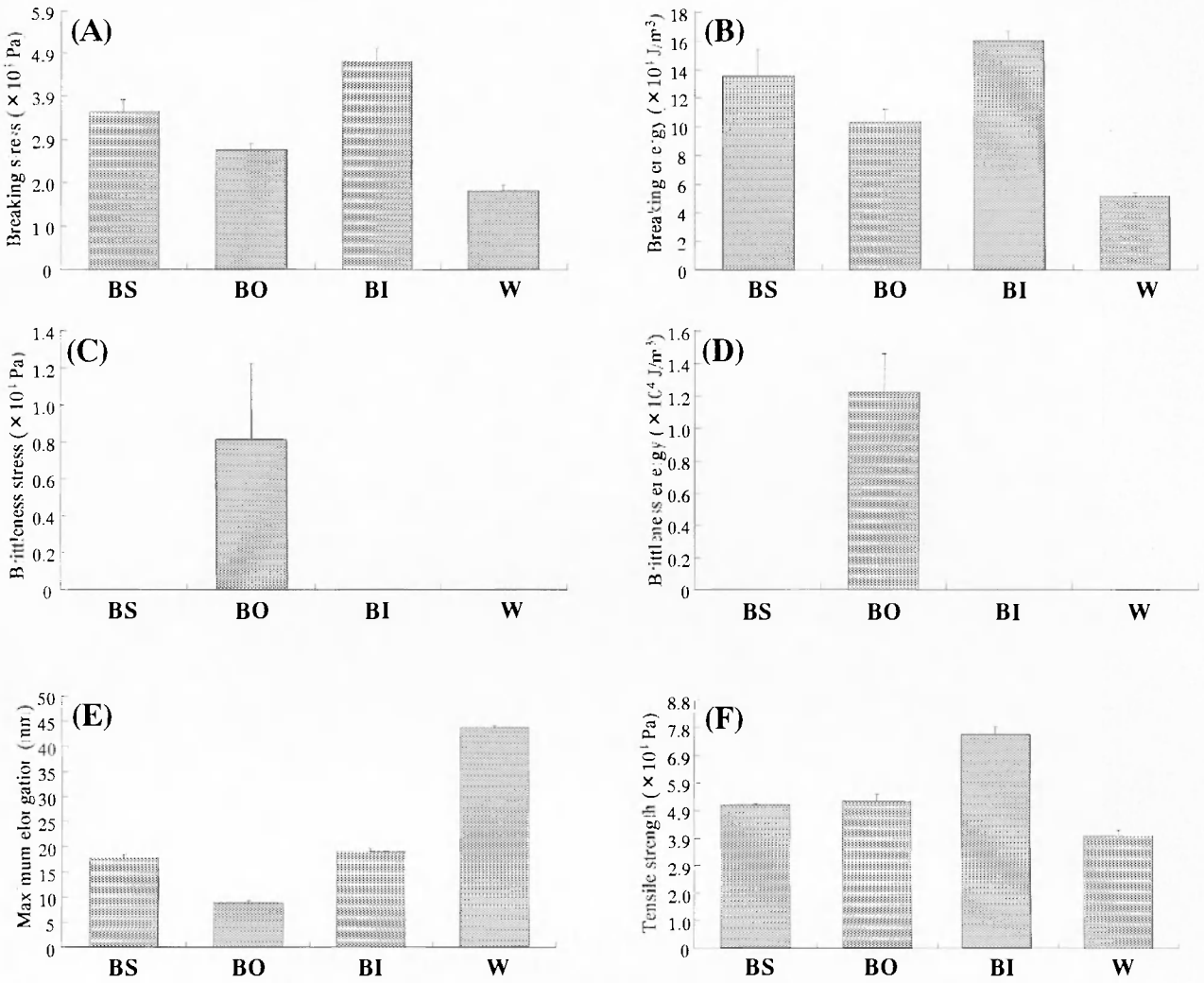

Fig. 4. Breaking and Tensile characteristics of buckwheat noodles and wheat noodles (udon noodles). Buckwheat noodles, which were prepared from various buckwheat flours, and udon noodles were analyzed. The abbreviations for the buckwheat samples were the same as in Fig. 1.

Table 2. Eigen vector, factor loading, eigen value and contribution extracted from principal component analysis of six characters for four samples

Character	Eigen vector		Factor loading	
	PC1	PC2	PC1	PC2
Breaking stress	0.5377	−0.1404	0.9751	−0.2211
Breaking energy	0.5434	−0.0466	0.9855	−0.0733
Brittleness stress	−0.0255	0.6311	−0.0463	0.9941
Brittleness energy	−0.0255	0.6311	−0.0463	0.9941
Maximum elongation	−0.3870	−0.4221	−0.7017	−0.6649
Tensile strength	0.5143	−0.0590	0.9326	−0.0929
Eigen value			3.29	2.48
Cumulative contribution (%)			54.81	96.17

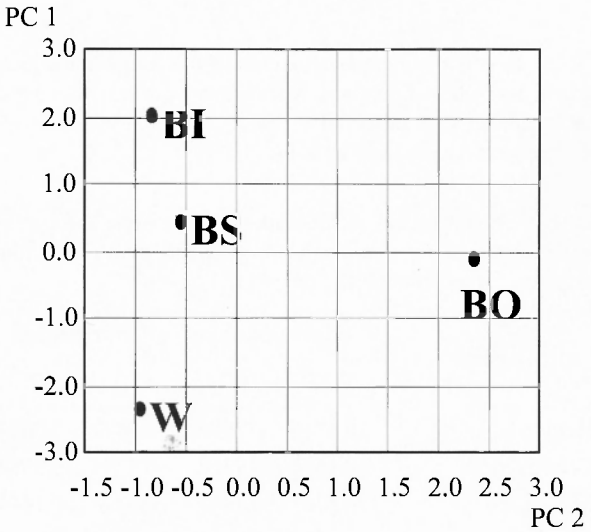

Fig. 5. Principal component analysis of various buckwheat noodles flours and wheat flour with respect to their breaking and tensile characteristics as observed in Fig. 4. The abbreviations for the samples were the same as in Fig. 1.

and tensile analyses can clearly exhibit a variation among the four noodles made from the inner-layer flour, outer-layer flour, whole flour of buckwheat, and wheat flour. Therefore, in a similar fashion to Figs. 2 and 3, Fig. 5 suggests that principal component analysis can demonstrate preference mapping for buckwheat foods, especially buckwheat noodles which are made from various buckwheat flours, e.g., sarashina-soba and inaka-soba.

Figure 6 and Table 3 shows the classification of various buckwheat noodles by principal component analysis with respect to their rupture and tensile. Interestingly enough, the buckwheat noodles were classified into three groups, i.e., hand-made buckwheat noodles (te-uti-men) (group 1), commercial dried buckwheat noodles (kan-men) (group 2) and commercial precooked buckwheat noodles (yude-men) (group 3) (Fig. 6). Furthermore, Fig. 6 also shows that nine noodles made from Tartary buckwheat (A-4, 5, 6, 7, B-9, 10, 11, 12, 13) did not form a group, but they belonged to either goup 1 or goup 2.

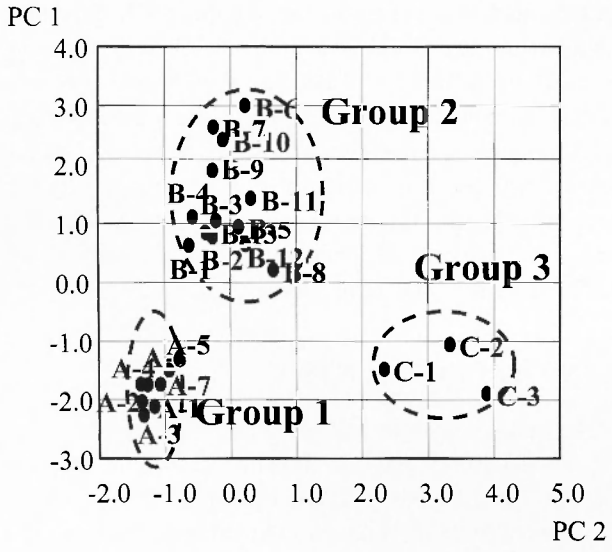

Fig. 6. Principal component analysis of various noodles, made from buckwheat flour and wheat flour, with respect to their breaking and tensile characteristics. The results of PC analyses were plotted based on the breaking and tensile (data not shown). PC analysis classified the noodles examined into three groups (Groups 1 to 3). Noodle A, which belonged to group 1 were hand-made buckwheat noodles (teuchi-men), noodles B of group 2 were commercial dried buckwheat noodles (kan-men), and noodles C of group 3 were commercial precooked buckwheat noodles (yude-men). A-4, 5, 6, 7, B-9, 10, 11, 12, 13 were noodles made from Tartary buckwheat flour.

Figure 7 and Table 4 shows the classification of various commercial dried buckwheat noodles (kan-men) by principal component analysis with respect of their rupture

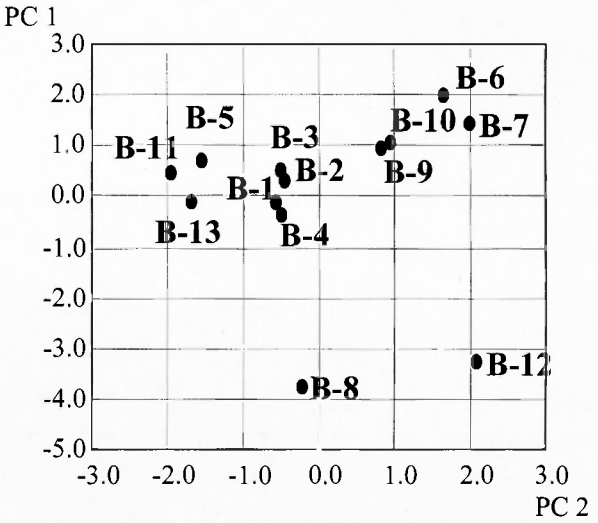

Fig. 7. Principal component analysis of various, commercial dried buckwheat noodles (kan-men). The abbreviations for the samples were the same as in Fig. 6. The dough-binder of the noodles, made from common buckwheat, B-1 to B-4 was wheat flour; the binder of B-6, yam tube flour; the binder of B-7, ground tea; and the binder of B-8, sea weed (Fu-nori). The noodles B-5 were made from common buckwheat by special noodle-making technology. The noodles B-9 to 13 were made from both Tartary buckwheat and common buckwheat.

Table 3. Eigen vector, factor loading, eigen value and contribution extracted from principal component analysis of six characters for twenty-three samples

Character	Eigen vector		Factor loading	
	PC1	PC2	PC1	PC2
Breaking stress	0.5525	0.1467	0.9473	0.2068
Breaking energy	0.5491	0.1268	0.9415	0.1788
Brittleness stress	−0.1950	0.6520	−0.3344	0.9192
Brittleness energy	−0.1572	0.6259	−0.2696	0.8823
Maximum elongation	0.2859	0.3758	0.4901	0.5298
Tensile strength	0.4987	−0.0653	0.8550	−0.0921
Eigen value			2.94	1.99
Cumulative contribution (%)			48.99	82.12

Table 4. Eigen vector, factor loading, eigen value and contribution extracted from principal component analysis of six characters for thirteen samples

Character	Eigen vector		Factor loading	
	PC1	PC2	PC1	PC2
Breaking stress	0.4612	0.3856	0.7819	0.5306
Breaking energy	0.2651	0.6147	0.4494	0.8459
Brittleness stress	−0.5441	0.2148	−0.9225	0.2956
Brittleness energy	−0.5431	0.2214	−0.9208	0.3047
Maximum elongation	0.3535	−0.3395	0.5993	−0.4672
Tensile strength	0.0320	0.5128	0.0542	0.7057
Eigen value			2.87	1.89
Cumulative contribution (%)			47.91	79.47

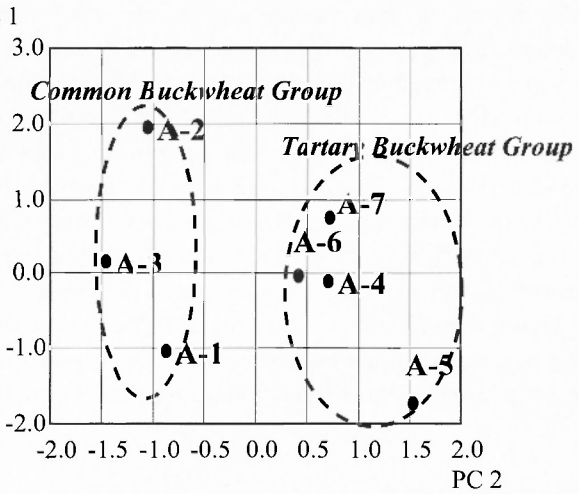

Fig. 8. Principal component analysis of various hand-made buckwheat noodles (te-uti-men). The abbreviations for the samples were the same as in Fig. 6.

Table 5. Eigen vector, factor loading, eigen value and contribution extracted from principal component analysis of six characters for seven samples

Character	Eigen vector		Factor loading	
	PC1	PC2	PC1	PC2
Breaking stress	0.5159	−0.1995	0.8497	−0.2450
Breaking energy	0.6378	0.2676	1.0506	0.3286
Brittleness stress	0.3520	0.2087	0.5799	0.2563
Brittleness energy	0.3520	0.2087	0.5799	0.2563
Maximum elongation	−0.2644	0.5566	−0.4355	0.6835
Tensile strength	−0.0964	0.7012	−0.1588	0.8611
Eigen value			2.71	1.51
Cumulative contribution (%)			45.22	70.35

and tensile analyses. Principal component analysis shows that various dry-type buckwheat noodles may be mainly classified based on the the noodle-binder or additives used in the preparation of the noodles, i.e., wheat flour, yam flour (Chinese yam, *Dioscorea batatas*), sea weed (fu-nori, *Gloiopeltis* spp.) and ground tea.

Figure 8 and Table 5 shows the classification of various hand-made buckwheat noodles (te-uti-men) by principal component analysis with respect to their rupture and tensile analyses. Principal component analysis shows that various hand-made buckwheat noodles were classified into two groups, i.e., common buckwheat noodle group and Tartary buckwheat noodles. In a previous paper (Ikeda et al., 2003), we have shown a striking difference in mechanical characteristics between common buckwheat and Tartary buckwheat. The present finding (Fig. 8) agrees well with our previous findings (Ikeda et al., 2003).

There are many types of buckwheat foods worldwide. Noodles made from buckwheat flour-water dough are the most popular food made from buckwheat in Japan. In view of their cooking and processing characteristics, a great deal of attention has been paid to the palatability and acceptability of different buckwheat foods including noodles. There is a large variety of buckwheat noodles in Japan. Clarifying consumer preference for the different types of noodles is an interesting subject. On the other hand, increasing attention has been paid to Tartary buckwheat products. We have shown a marked difference in the mechanical characteristics between common and Tartary buckwheat (Fig. 8). Our findings will hopefully stimulate further development in the direction of new products from Tartary buckwheat. Finally, we conclude that multivariate analysis, i.e., principal component analysis and cluster analysis, with respect to mechanical characteristics can demonstrate preference mapping for buckwheat foods. The present findings show a scientific basis for clarifying the palatability and acceptability of buckwheat foods including noodles.

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Nutritional characteristics of minerals in Tartary buckwheat

Sayoko IKEDA[1*], Kazue TOMURA[1], Lufa LIN[2] and Ivan KREFT[3]

[1] *Faculty of Nutrition, Kobe Gakuin University, Nishi-ku, Kobe 651-2180, Japan*
[2] *Shanxi Academy of Agricultural Sciences, Taiyuan, Shanxi, Province, China*
[3] *Biotechnical Faculty, University of Ljubljana, 1001 Ljubljana, Slovenia*

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Key words: Tartary buckwheat, essential mineral, essential trace elements

ABSTRACT

The composition of eight minerals, i.e., iron, zinc, copper, manganese, calcium, magnesium, potassium, phosphorus in Tartary buckwheat flour was analyzed; and the release of the minerals in soluble form after in vitro enzymatic digestion of the Tartary buckwheat flour was evaluated. The results were compared with those of common buckwheat. A variation in the mineral composition among the Tartary buckwheat flours examined was found. One way analysis of variance showed a significant ($P<0.05$) difference in the content of manganese and potassium between common and Tartary buckwheat, whereas no significant difference in the other mineral contents was found between the two buckwheat species. The present study has shown that the largest part of the zinc, copper and potassium is released in soluble form from the Tartary buckwheat flour after enzymatic digestion. The nutritional implications for the present findings are discussed.

INTRODUCTION

Buckwheat (*Fagopyrum* spp.) is an important human food in some area of the world. It is consumed in many countries, including Japan, China, other Asian countries and many European countries, including Slovenia and Italy (Ikeda and Ikeda, 1999, 2003). In view of some of the beneficial effects for human health, such as beneficial effects on blood pressure and on cholesterol metabolism (He et al., 1995) and a low glycemic index (Jenkins, 1981; Foster-Powell and Miller, 1995), increased attention has been currently paid to buckwheat as a functional food (Bonafaccia and Kreft, 1998; Mazza, 1998). In this connection, it is thought that buckwheat intake may lead to lowering of high blood pressure. Some factors, e.g., rutin and angiotensin converting enzyme I inhibitor are thought to be involved in such a beneficial effect. Minerals, such as potassium, may also be associated with lowering blood pressure, but the exact mechanism remains obscure. Thus, clarifying the characteristics of buckwheat minerals is a subject of great interest. We have conducted several studies on the nutritional function of essential minerals in buckwheat and its products (Ikeda and Yamashita, 1994; Ikeda, 1996; Ikeda et al., 2001, 2002, 2003). The nutritional characteristics of essential minerals in buckwheat, however, have not been fully clarified.

There are two cultivated species of buckwheat i.e., common (*F. esculentum* Moench) and Tartary (*F. tataricum* Gaertner). Common buckwheat is widely utilized worldwide, whereas Tartary buckwheat is utilized in limited regions including China, Nepal and Bhutan. On the other hand, many beneficial effects of Tartary buckwheat on human health have been suggested (Lin et al., 1998). In view of such a suggestion, interest in buckwheat is currently rapidly growing. In fact, various Tartary buckwheat products, including noodles and roasted grain tea, have become available in Japan.

The present study was undertaken to analyze the composition of eight essential minerals, i.e., iron, zinc, copper, manganese, calcium, magnesium, potassium and phosphorus in Tartary buckwheat flour, and to clarify any changes in the amount of the eight minerals in their soluble form after a vitro enzymatic digestion of the flour. It also compared the characteristics of these minerals in Tartary and common buckwheat.

MATERIALS AND METHODS

Materials

Four Tartary buckwheat samples, one Japanese and three Chinese, were analyzed in this study. The Japanese sample, i.e., var. Hokkei No. 1 was kindly provided by Hokkaido National Agriculture Experiment Station (Hokkaido, Japan). The three Chinese samples that were selected for this study were var. Hei Ku Qiao, var. Hui Ku Qiao and var. Shoungang Bendi Ku Qiao. The first three samples were provided as seed, however, the last one was provided as flour. The Tartary buckwheat seeds were milled to fine flour in our laboratory according to a method as described previously (Ikeda et al., 2001, 2002).

* Corresponding author

Determination of essential minerals, protein and moisture

Eight essential minerals, i.e., iron, zinc, copper, manganese, calcium, magnesium, potassium and phosphorus, were assayed in this study. The essential minerals, except for phosphorus, of the Tartary buckwheat flour samples were determined with a Hitachi Z-5300 polarized Zeeman atomic absorption spectrophotometer (Hitachi Ltd., Hitachi-naka, Japan). The phosphorus was assayed using the colorimetric method of Fiske and Subbarow (1925). Prior to determination of the total content of these essential minerals, the buckwheat flour samples were wet-ashed with sulfuric acid and 30% hydrogen peroxide. The protein content, estimated by N X 6.25, was analyzed by the micro-Kjeldahl method (AOAC, 1984). The moisture content was determined by drying for 3 hr at 135°C (RCSTAJ, 2000).

In vitro proteolytic digestion

The raw Tartary buckwheat samples were subjected to in vitro enzymatic digestion with α-amylase, pepsin plus pancreatin according to the method described previously (Ikeda, 1984, 1990; Ikeda and Murakami, 1995; Ikeda et al., 2003). Firstly, α-amylase digestion was performed in 0.02 M Tris-HCl buffer (pH 7.0) which contained 560 units of α-amylase for 30 min at 37°C. Immediately after the α-amylase digestion, an appropriate volume of 2 N HCl (pH 1.0) was added to the digestion mixtures to adjust pH 1.0 to 1.3. Pepsin digestion was subsequently performed in 0.06 N hydrochloric acid for 3 hr at 37°C with an enzyme-to-protein weight ratio of 1:100. Immediately after the peptic digestion, an appropriate volume of 2 M Tris-HCl buffer (pH 8.0) was added to the digestion mixtures to adjust the pH to 8.0. Toluene was then added to the buffer to prevent any growth of microorganisms, to a final concentration of 0.0013%. A pancreatin solution with deoxycholate was then added to their digestion mixtures at an enzyme-to-protein weight ratio of 1:20, and it was subsequently incubated for an additional 20 hr at 37°C (pH 8.0). The final concentration of Tris-HCl buffer in the digestion mixtures was 0.2 M. Deoxycholate was added to the digestion medium to a final concentration of 0.1% to accelerate digestion of the fatty components. Immediately after the digestion, the suspensions were placed in an ice-cold vessel to diminish enzymatic action and were then clarified by centrifugation at 10,000 rpm for 20 min. The supernatants obtained were subjected to analysis of the minerals.

Statistical analysis

The data obtained were subjected to analysis of variance and the significance of means was tested by the Tukey's multiple range test (Steel and Torrie, 1980). One way analysis of variance was performed using a personal computer with the program Excel Statistics (Esumi Co. Ltd., Tokyo, Japan).

RESULTS AND DISCUSSION

Essential mineral composition in Tartary buckwheat and its nutritional contribution

Table 1 shows the composition of the eight essential minerals, i.e., iron, zinc, copper, manganese, calcium, magnesium, potassium and phosphorus, and the total protein content in Tartary and common buckwheat flour. There were variations in the content of some of the minerals among the varieties examined (Table 1). The iron content per 100 g dry matter (DM) of the Tartary buckwheat flour ranged from 3.67 to 7.08 mg with an average of 5.14 mg; the zinc content, from 1.26 to 2.83 mg with an average of 1.70 mg; the copper, from 0.30 to 0.66 mg with an average of 0.44 mg; the manganese, from 0.82 to 1.22 mg with an average of 1.06 mg; the calcium, from 12.4 to 47.7 mg with an average of 36.3 mg; the magnesium, from 171 to 268 mg with an average of 228 mg; the potassium, from 300 to 397 mg with an average of 363 mg; and the phosphorus, from 226 to 430 mg with an average of 371 mg. A one way analysis of variance showed that there was a significant ($P<0.05$) difference in the content of manganese and potassium between common and Tartary buckwheat, whereas no significant difference ($P>0.05$) in the other mineral contents was found between the two buckwheat species. Therefore, these finding suggest that there may be a difference in the content of manganese and potassium between common and Tartary buckwheat, although further analysis may be required to verify this assumption.

Although an exact reason for the observed variations in the content of the essential minerals among the buckwheat samples remains obscure, the content of the minerals in buckwheat flour may be generally influenced by factors such as genetic composition, environmental conditions and processing methods. From the standpoint of mineral nutrition, it is of great interest to determine which factor may be the most important one that was responsible for the observed variations in the mineral contents of buckwheat. Namely, Tartary buckwheat with a high content of minerals might be a potential source of these minerals. Interestingly, there were some characteristics of the mineral content between Tartary and common buckwheat, and between the Japanese samples and Chinese samples, i.e., Tartary buckwheat, as examined by one way analysis of variance, contained a significantly lower content of both manganese and potassium when compared with the common buckwheat samples (Table 1). A significantly ($P<0.01$) higher content of both zinc and copper were found in the Japanese Tartary and com-

Table 1. Contents of eight essential minerals and total protein in the flour of Taratry and common buckwheat[1)]

Buckwheat samples	Fe	Zn	Cu	Mn	Ca	Mg	K	P	Protein
	mg/100 g DM								g/100 g DM
[Tartary buckwheat]									
—Japanese sample—									
Hokkei No. 1	3.67^{d}	2.83^{a}	0.66^{a}	1.03^{e}	12.4^{g}	226^{d}	397^{d}	428^{b}	11.47^{a}
—Chinese samples—									
Hei Ku Qiao	7.08^{a}	1.34^{de}	0.41^{d}	1.18^{d}	47.7^{a}	268^{b}	386^{d}	399^{c}	9.87^{b}
Hui Ku Qiao	4.88^{bc}	1.38^{d}	0.39^{d}	1.22^{d}	43.9^{b}	247^{c}	368^{e}	430^{b}	12.00^{a}
Shoungang Bendi Ku Qiao	4.92^{b}	1.26^{e}	0.30^{e}	0.82^{f}	41.0^{c}	171^{e}	300^{f}	226^{d}	6.51^{e}
Means [N=4]	5.14 ±1.42	1.70 ±0.75	0.44 ±0.15	1.06 ±0.18	36.3 ±16.1	228 ±42	363 ±44	371 ±98	9.96 ±2.47
CV[2)] [%]	27.6	44.1	34.1	17.0	44.4	18.4	12.1	26.4	24.8
[Common buckwheat[3)]]									
Kitawase	3.37^{d}	2.72^{b}	0.54^{c}	2.43^{a}	15.1^{f}	254^{c}	453^{c}	444^{b}	7.68^{d}
Hashikami-wase	4.48^{c}	2.76^{ab}	0.59^{b}	1.52^{c}	25.8^{d}	280^{a}	511^{a}	500^{a}	8.32^{cd}
Hitachi-akisoba	4.59^{bc}	2.54^{c}	0.52^{c}	1.85^{b}	20.0^{e}	257^{c}	471^{b}	489^{a}	9.11^{bc}
Means [n=3]	4.15 +0.67	2.67 ±0.12	0.55 ±0.04	1.93 ±0.46	20.3 ±5.4	264 ±14	478 ±30	478 ±30	8.37 ±0.72
CV[2)] [%]	16.1	4.5	7.3	23.8	26.6	5.3	6.3	6.3	8.6

[1)] Values are means±S.D. [n=4] on a dry weight basis. Values within a column that do not share a common superscript are significantly different at $p<0.01$.
[2)] CV, coefficient of variation.
[3)] Ikeda et al. (2001, 2002)

mon buckwheat than what was found in the Chinese Tartary buckwheat (Table 1), whereas a significantly ($P<0.01$) higher content of calcium was found in the Chinese Tartary buckwheat than what was found in either Japanese Tartary or common buckwheat. The content of iron and magnesium showed no significant ($P>0.01$) difference between Tartary and common buckwheat, or between the Japanese and Chinese samples. In addition, the var. Shoungang Bendi of the Chinese sample contained the lowest level of zinc, copper, manganese, magnesium, potassium and phosphorus among all the samples examined, although it should be noted that the flour sample of var. Shoungang Bendi, as indicated in the Materials and Methods, was prepared by a different milling method.

There were also variations in the total protein content among the varieties examined. The protein content, per 100 g dry matter (DM), of Tartary buckwheat flour ranged from 6.51 to 12.00 mg, with an average of 9.96 mg. The protein content showed that there was no variation between Tartary and common buckwheat, and between the Japanese and Chinese samples.

The present study has shown that Tartary buckwheat flour contains various kinds of essential minerals (Table 1). In this connection, recommended dietary allowances for essential minerals have recently been established in many countries. The Ministry of Health and Welfare in Japan (1999) have established the recommended dietary reference intakes for essential minerals (RDA). Based on both the analytical data in Table 1 and the RDA (MHWJ, 1999), we evaluated the nutritional contribution of Tartary buckwheat flour for the eight minerals. Our evaluation suggested that 100 g of Tartary buckwheat flour can provide approximately 37 to 45% of the iron, approximately 12 to 17% of the zinc, approximately 21 to 27% of the copper, approximately 23 to 31% of the manganese, approximately 4 to 5% of the calcium, approximately 62 to 83% of the magnesium, approximately 16% of the potassium and approximately 46% of the phosphorus required of the RDA. Thus Tartary buckwheat flour can provide a potential source for iron, zinc, copper, manganese, magnesium, potassium and phosphorus, but not for calcium, to meet the requirements of the RDA. This finding agrees with similar previous findings for common buckwheat flour (Ikeda et al., 2001, 2002).

Table 2. Proportion to eight water-soluble minerals of the flours of Tartary buckwheat[1]

Buckwheat samples	Fe	Zn	Cu	Mn	Ca	Mg	K	P
	μg/100 g food				mg/100 g food			
	(%)				(%)			
—Japanese sample—								
Hokkei No.1	245±13 (6.7)	1120+13 (39.6)	198±4 (29.8)	208±5 (20.1)	4.7±0.2 (37.7)	60.5±0.9 (26.7)	198±3 (49.9)	122±2 (28.5)
—Chinese samples—								
Hei Ku Qiao	120±9 (1.9)	476±27 (40.7)	118+6 (33.3)	203+13 (19.8)	12.4+0.3 (29.8)	61.2+0.4 (26.2)	167±1 (49.8)	106±1 (30.6)
Hui Ku Qiao	125+8 (3.0)	587+23 (49.7)	143+7 (43.0)	241+12 (23.1)	10.8+0.2 (28.6)	62.8+1.4 (29.7)	176±2 (55.8)	121+1 (32.8)
Shoungang Bendi Ku Qiao	172±7 (4.1)	618+32 (57.4)	98±7 (37.9)	183±8 (26.1)	10.4±0.2 (29.8)	64.6+0.9 (44.2)	188+2 (73.1)	110+2 (56.8)
Means [n=4]	166±58 (3.9±2.1)	700±286 (46.9±8.4)	139±43 (36.0±5.7)	209±24 (22.3+3.0)	9.6±3.4 (31.5+4.2)	62.3±1.8 (31.7+8.5)	182±14 (57.2±11.0)	115±8 (37.2±13.2)
CV[2] [%]	34.9 (53.8)	40.9 (17.9)	30.9 (15.8)	11.5 (13.5)	35.4 (13.3)	2.9 (26.8)	7.7 (19.2)	7.0 (35.5)

[1] Values are means±S.D. on a wet weight basis [n=4]. Values in parentheses indicate means of proportion (%) of each water-soluble component to the total content of buckwheat flour.
[2] CV, coefficient of variation.

Distribution of essential minerals in Tartary buckwheat

Table 2 shows the amount of water-soluble minerals, i.e., iron, zinc, copper, manganese, calcium, magnesium, potassium and phosphorus in Tartary buckwheat flour. There was variation in all of the water-soluble essential minerals found. A relatively high proportion of water-soluble minerals were found for potassium, zinc, phosphorus, copper, magnesium, calcium and manganese, whereas a low proportion was found for iron.

Essential minerals released after in vitro enzymatic digestion of Tartary buckwheat

Table 3 shows the amounts of eight essential minerals, i.e., iron, zinc, copper, manganese, calcium, magnesium, potassium and phosphorus, released in soluble form after an α-amylase, pepsin plus pancreatin digestion of the Tartary buckwheat flour and their proportion (%) as compared to the original total content in the flour. A large variation in the proportion of the minerals released on enzymatic digestion was found among the eight mineral. A higher proportion of the released amount following enzymatic digestion was found for zinc, copper and potassium, whereas a lower proportion of the released amount after enzymatic digestion was found for iron, calcium and manganese. These finding were in agreement with our previous results from common buckwheat flour (Ikeda et al., 2001, 2002). A higher proportion (%) of the zinc was released on enzymatic digestion of the Tartary buckwheat flour (Table 3) as compared with zinc that is present in rice (Ikeda, 1990).

In general, information on both the amount of food minerals and their bioavailability in the intestinal tract is important for evaluation of the exact mineral adequacy of diets. Thus clarifying the bioavailability of food minerals is a research subject of great current interest. It appears that the bioavailability of minerals in foods for intestinal absorption may be closely associated with their solubility in the intestinal tract. In this connection, we have reported on an enzymatic digestion method evaluating the availability of minerals with different foods, and the characteristics of their minerals (Ikeda, 1984, 1990; Ikeda et al., 1990; Ikeda and Murakami, 1995). The present study on Tartary buckwheat (Table 3) has shown that enzymatic digestion enables a large proportion, of over 65%, of three minerals, i.e., zinc copper and potassium, to be released in soluble form, but much smaller proportions of three other minerals, i.e., iron, calcium and manganese. These findings agree with our previous results from common buckwheat flour (Ikeda et al., 2001, 2003). In addition, low levels of the released amounts of manganese, calcium and magnesium, after enzymatic digestion were found (Table 3), as compared with those of the water-soluble minerals (Table 2). Therefore, enzymatic digestion may not enable these minerals to be release in a soluble form. On the other hand, there may be various, endogenous factors affecting the solubility of minerals after digestion. Characterization of such factors will be an

Table 3. Proportion of their original total contents of the amounts of eight essential minerals released as soluble forms after the pepsin plus pancreatin digestion of the flours of Tartary buckwheat[1)]

Buckwheat samples	Fe	Zn	Cu	Mn	Ca	Mg	K	P
		μg/100 g food				mg/100 g food		
		(%)				(%)		
—Japanese sample—								
Hokkei No. 1	494±17 (13.5)	1730+36 (61.2)	426±10 (64.1)	94±2 (9.1)	1.54+0.05 (12.4)	55.5+1.5 (24.5)	168±3 (42.3)	159+1 (37.3)
— Chinese samples —								
Hei Ku Qiao	639±12 (10.4)	861±23 (73.6)	210±9 (59.3)	210±9 (20.4)	6.42+0.13 (15.5)	62.2±0.7 (26.7)	254±1 (73.1)	142±3 (41.0)
Hui Ku Qiao	696±25 (16.6)	959+17 (81.3)	264±10 (79.0)	189+3 (18.1)	5.45±0.12 (14.5)	67.6±0.7 (31.9)	239±3 (75.5)	163±2 (44.3)
Shoungang Bendi Ku Qiao	689±27 (16.3)	828+13 (76.9)	191+11 (73.8)	208±6 (29.7)	6.72±0.13 (19.1)	57.2+1.2 (39.1)	195+2 (76.0)	112+3 (57.6)
Means [n=4]	630+94 (14.2+2.9)	1095±427 (73.3±8.6)	273±107 (69.1±9.0)	175±55 (19.3±8.5)	5.03±2.39 (15.4+2.8)	60.6±5.5 (30.6±6.5)	212+37 (66.7+16.3)	144±23 (45.1±8.8)
CV[2)] [%]	14.9 (20.4)	39.0 (11.7)	39.2 (13.0)	31.4 (44.0)	47.5 (18.2)	9.1 (21.2)	17.5 (24.4)	16.0 (19.5)

[1)] Values are means ±S.D. on a wet wight basis [n=4]. Values in parentheses indicate means of proportion (%) of each mineral released on the digestion of buckwheat flour to each total mineral contents.
[2)] CV, coefficient of variation.

interesting subject in the future.

In conclusion, the present findings (Table 1) have shown that Tartary buckwheat flour contains high levels of iron, zinc, copper, manganese, magnesium, potassium and phosphorus, but low levels for calcium. Our evaluation suggests that Tartary buckwheat flour can be an important source of the above seven minerals, providing approximately 10 to 80% of the RDA for these seven minerals, with calcium being an exception. On the other hand, the present findings (Table 3) have shown that enzymatic digestion enables large proportions of three minerals, i.e., zinc, copper and potassium, to be released in soluble forms, but smaller proportions of three other minerals, i.e., iron, calcium and manganese. These findings (Table 3) suggest the possibility that the three minerals, i.e., zinc, copper and potassium, in Tartary buckwheat flour may be available for intestinal absorption. However, the detailed mechanism involved remains unclear. Research is currently in progress in our laboratory to characterize the minerals in common and Tartary buckwheat and their products in relation to their bioavailability.

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Mechanical characterization of buckwheat products made with waxy-wheat flour

Yuya ASAMI[1], Chikako KIRIBUCHI-OTOBE[2] and Kiyokazu IKEDA[3]*

[1] *Kyoto College of Nutritional & Medical Sciences, Ukyo-ku, Kyoto 616-8376, Japan*
[2] *National Institute of Crop Science, 2-1-18, Kannondai, Tukuba, Ibaraki 305-8518, Japan*
[3] *Faculty of Nutrition, Kobe Gakuin University, Nishi-ku, Kobe 651-2180, Japan*

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ABSTRACT

The present study was conducted to incorporate waxy wheat flour as an additive to buckwheat products and to characterize buckwheat products developed with the addition of waxy wheat flour. Buckwheat dough and noodles with waxy wheat flour were shown to have unique mechanical characteristics. The present study suggests the possibility of development of buckwheat products made with waxy wheat flour as new buckwheat products.

INTRODUCTION

Buckwheat (*Fagopyrum* spp.) is an important crop in some regions of the world (Kreft et al., 2003; Ikeda, 2002). Buckwheat flour contains many essential nutrients including protein (Ikeda et al., 1991) and minerals (Ikeda and Yamashita, 1994) at high levels. Thus, buckwheat flour contributes as an important dietary source of these essential nutrients.

There is a large variety of buckwheat foods produced on a global basis (Ikeda, 2002). Noodles made from buckwheat flour are popular in some countries such as Japan, Korea and China. As buckwheat flour has low cohesiveness, dough-binders, such as wheat flour and eggs, are usually added in making the buckwheat noodles. Increasing attention has been paid to the palatability and acceptability of buckwheat products from the perspective of their cooking and processing ability. It is hoped that new buckwheat products with increased palatability and acceptability will be developed. In this connection, wheat with a waxy endosperm character (waxy wheat) has been bred (Kiribuchi-Otobe et al., 1997). However, waxy wheat still has not been widely utilized. The addition of waxy wheat flour as an additive to buckwheat products might lead to the development of new buckwheat products with appropriate palatability and acceptability.

The present study was conducted to evaluate the incorporation waxy wheat flour as an additive to buckwheat products and to characterize buckwheat products which contained waxy wheat flour.

MATERIALS AND METHODS

Materials

Buckwheat grain (*F. esculentum* Moench var. Kitawase soba), which was harvested in Hokkaido in 2002, was used in this study. The buckwheat grain was dehulled with an electrically driven dehuller (Mini-Dappu FC2K, Ohtake Seisaku-sho, Aichi, Japan). The buckwheat seed obtained was milled with a roller mill (Quadrumat junior, Model No. 279002, Brabender OHG Duisburg, Germany) fitted with a 231 μm sieve. The waxy wheat flour sample (*Triticum aestivum L.* var. Kanto-mochi No. 124) used in this study was obtained from the National Institute of Crop Science (Ibaraki, Japan). Waxy wheat grain was milled with Buhler test mill (Germany) fitted with a 357 μm-sieve. Non-waxy wheat (medium flour) used in this study was a commercial product.

Mechanical measurements

The mechanical characteristics of the buckwheat products were evaluated using two different mechanical analysis: texture analysis and tensile analysis. Texture analysis of the buckwheat dough was performed using a Reolometer RX-1600 (Iio Denki Co., Japan) according to the procedure previously described by Ikeda et al. (1997, 1999). The tensile analysis of the buckwheat noodles was preformed with a Rheometer RT-3005D using the procedure previously described (Ikeda and Asami, 2000). The preparation methods for the buckwheat noodles was performed using the method described previously (Ikeda and Asami, 2000). Mechanical measurements of the buckwheat products samples were repeated from five to ten times with the different samples.

* Corresponding author

Extraction and electrophoresis

Polyacrylamide gel electrophoresis with sodium dodecylsulfate (SDS-PAGE) was performed to allow comparison of the protein composition between the two buckwheat samples containing either waxy wheat flour or non-waxy wheat flour. The protein of the combined fraction was extracted using the procedure of Ikeda and Asami (2000). Briefly stated, the buckwheat flour, waxy wheat flour and non-waxy wheat flour, which were obtained above, were extracted with a ten fold (v/w) volume of 1% sodium dodecylsulfate for 1 hr at 20°C. After extraction, the suspension was centrifuged at 17,000×g for 20 min, and then 10 μl of the supernatants was subjected as the combined fraction of protein to SDS-PAGE. SDS-PAGE was performed according to the method of Laemmli (1970) in the presence of 2-mercaptoethanol. The protein was stained with Coomassie Brilliant Blue R-250 and then destained with 7% acetic acid solution. Bovine serum albumin (66 kDa), trypsinogen (24 kDa), β-lactoalbumin (189.4 kDa), and lysozyme (14.3 kDa) were used as molecular weight markers.

Scanning electron microscopy

Scanning electron microscopy was performed using a Hitachi S-2150 scanning electron microscopy to compare the structure of buckwheat noodles containing either waxy or non-waxy wheat flour. The buckwheat samples were prepared with glutaraldehyde according to a method reported previously (Ikeda et al., 2001).

Statistical analysis

Statistical analysis was conducted using a personal computer with the program Excel (Microsoft Co., USA).

RESULTS AND DISCUSSION

Mechanical characteristics of buckwheat products with waxy wheat flour

Figure 1 shows the textural characteristics of buckwheat dough with either waxy wheat flour or with non-waxy wheat flour. Considerably high adhesiveness (Fig. 1 (C)) and springiness (Fig. 1 (D)) were found in the buckwheat dough with the waxy wheat flour. On the other hand, relatively low hardness (Fig. 1 (A)) and chewiness (Fig. 1 (E)) were found with the buckwheat dough with waxy

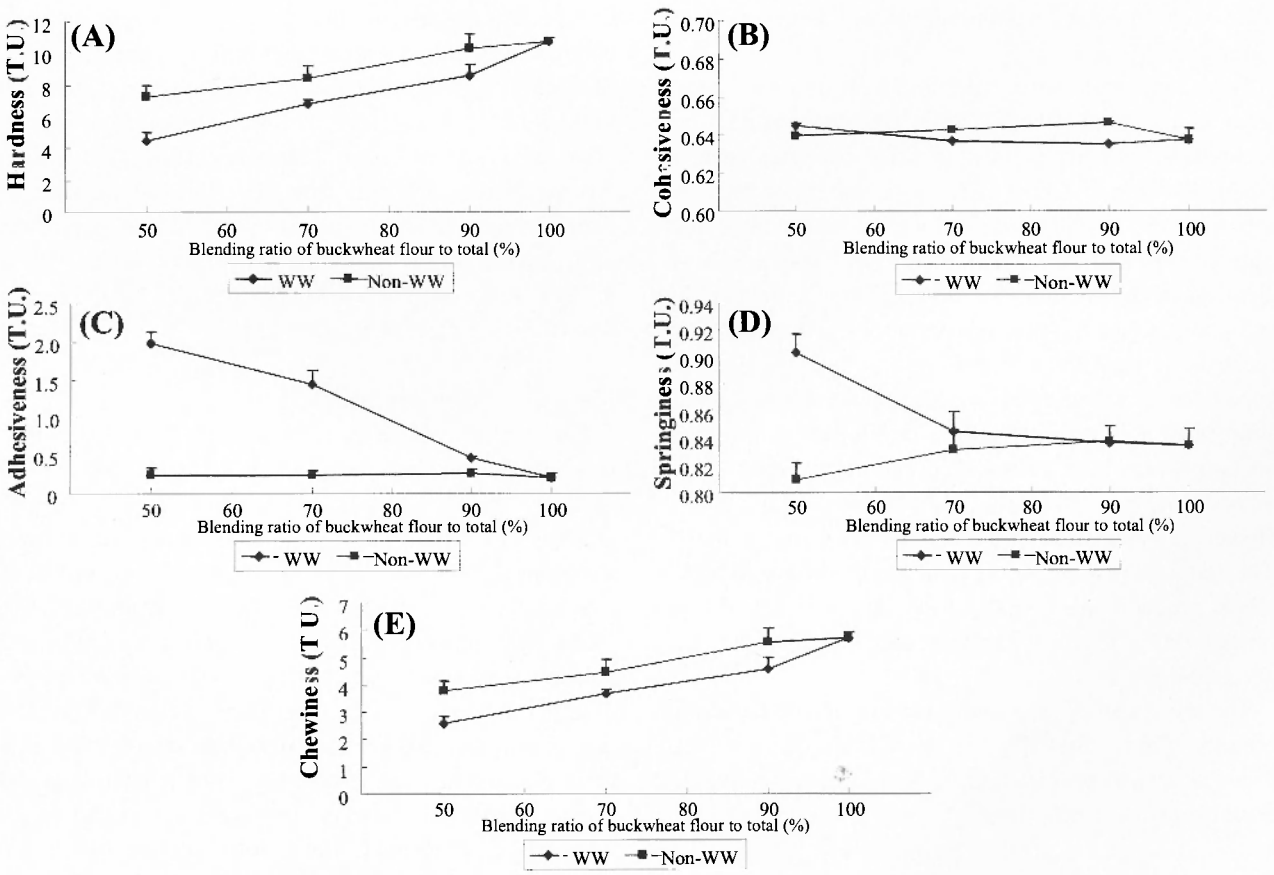

Fig. 1. Textural characteristics of buckwheat dough made with waxy wheat flour or with non-waxy wheat flour.
(A) hardness; (B) cohesiveness; (C) adhesiveness; (D) springiness; (E) chewiness.
Buckwheat doughs made with waxy wheat flour (WW) and with non-waxy wheat flour (Non-WW) were subjected to texture analysis.

wheat flour, as compared to the buckwheat dough with non-waxy wheat flour (Fig. 1 (A), (E)). Therefore, incorporation of waxy wheat flour as an additive to the buckwheat dough was shown to lead to enhancement in both adhesiveness and springiness (Fig. 1).

Figure 2 shows the tensile characteristics of buckwheat noodles made with either waxy wheat flour or with non-waxy wheat flour. Maximum elongation in the buckwheat noodles containing waxy wheat was higher than those having non-waxy wheat flour (Fig. 2 (A)). On the other hand, the tensile strength of the buckwheat noodles having waxy wheat flour was lower than those of with non-waxy wheat flour (Fig. 2 (B)). Thus incorporation of waxy wheat flour as an additive in buckwheat noodles was shown to lead to a reduction in tensile strength with an increased maximum elongation (Fig. 2).

Figure 3 shows principal component analysis with respect to the observed textural and tensile characteristics (Figs. 1 and 2) of buckwheat noodles with either waxy wheat flour or with non-waxy wheat flour. Principal component analysis (Fig. 3) showed that buckwheat noodles with waxy wheat flour exhibited unique mechanical characteristics as compared to buckwheat noodles containing non waxy wheat flour.

Protein components of buckwheat noodles with waxy wheat flour

Figure 4 shows the SDS-PAGE patterns of protein composition of buckwheat noodles with the addition of waxy wheat flour or with non-waxy wheat flour. Various protein components were found in the buckwheat flour (RBW in Fig. 4 (A) and (B)), with either waxy wheat flour (HWW and RWW in Fig. 4 (A) or with non-waxy wheat flour (HNWW and RNWW in Fig. 4 (B)). The specification of each protein component in raw buckwheat flour (RBW) by molecular weight was reported in a previous paper (Ikeda and Asami, 2000). As indicated by the respective circle (HRWW and HRNWW; RWW and RNWW in Fig. 4), there was variation in some of the protein components containing waxy wheat flour (HWW and RWW in Fig. 4 (A) or non-waxy wheat flour (HNWW and RNWW in Fig. 4 (B)). The observed variation in the protein composition ((A) and (B) in Fig. 4) may be associated with the observed mechanical characteristics (Figs. 1 and 2), but the exact mechanism which was involved remains uncertain.

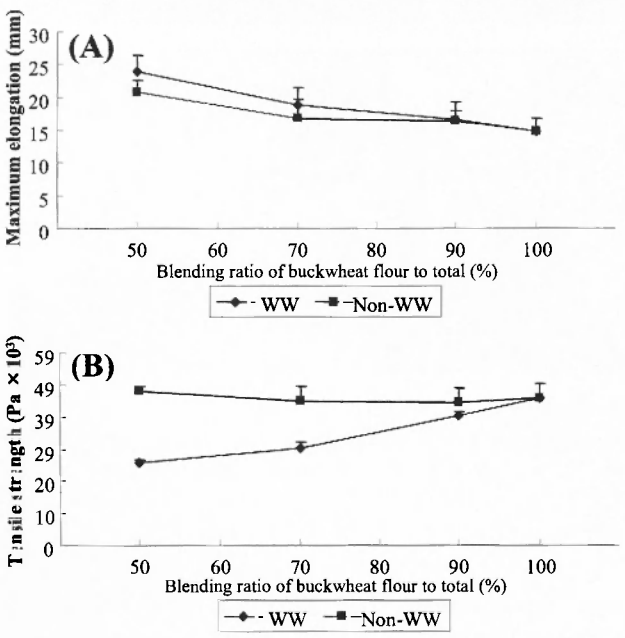

Fig. 2. Tensile characteristics of buckwheat noodles made with waxy wheat flour or with non waxy wheat flour.
(A) maximum elongation; (B) tensile strength.
Buckwheat noodles made with waxy wheat flour (WW) and with non-waxy wheat flour (Non-WW) were subjected for tensile analysis.

Scanning electron microscopy (SEM) of buckwheat noodles with waxy wheat flour

Figure 5 shows the SEM of noodles made from buckwheat flour alone ((A) and (D) in Fig. 5) buckwheat noodles made with the addition of waxy wheat flour ((B) and (E) in Fig. 5) or non-waxy wheat flour ((C) and (F) in Fig. 5). There was a marked variation in structure among the noodles made from buckwheat flour alone ((A) and (D) in Fig. 5), buckwheat noodles with the addition of waxy wheat flour ((B) and (E) in Fig. 5), and buckwheat noodles with the addition of non-waxy wheat flour ((C) and (F) in Fig. 5). There was difference in the SEM between noodles made from buckwheat flour alone ((A) and (D) in Fig. 5) and noodles made with buckwheat flour together with non-waxy wheat flour ((C) and (F) in Fig. 5). This difference in the SEM may be ascribable to the presence of the gluten molecule in the non waxy wheat flour, although the detailed information still

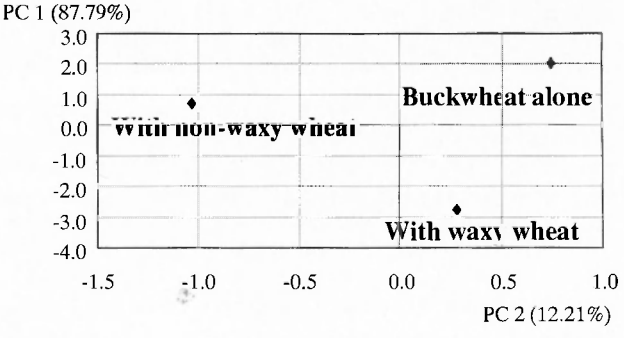

Fig. 3. Principal component analysis of the buckwheat products made with waxy wheat flour or with non-waxy wheat flour with respect to textural characteristics and tensile characteristics observed in Figs. 1 and 2. The major components of PC 1 consisted of hardness and the major components of PC 2 consisted of springiness and chewiness.

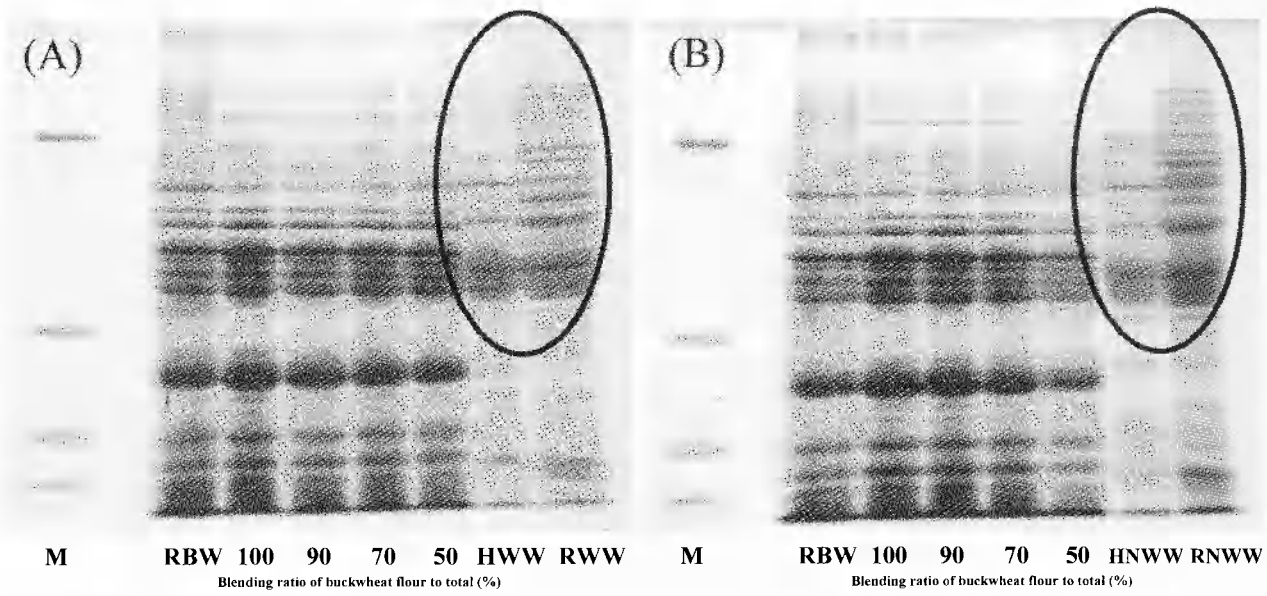

Fig. 4. SDS-PAGE patterns of protein component of buckwheat noodles made with waxy and non-waxy wheat flour.
RBW: raw buckwheat flour, HWW: boiled (95°C, 1.5 min) waxy wheat, RWW: raw waxy wheat flour, HNWW: boiled (95°C, 1.5 min) non-waxy wheat, RNWW: raw non-waxy wheat. Preparation of the noodles included boiling them.

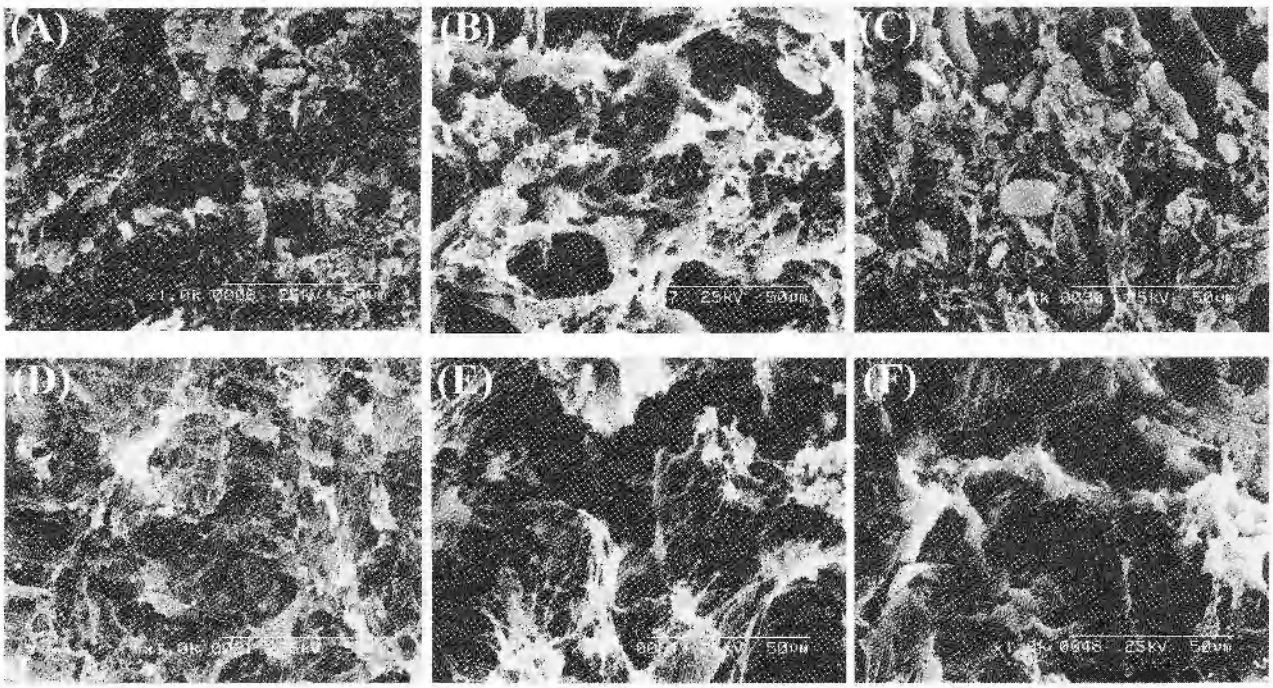

Fig. 5. Scanning electron microscopy of buckwheat noodles made with waxy and non-waxy wheat.
(A) a cross-section of buckwheat noodles made from buckwheat flour alone; (B) a cross-section of buckwheat noodles made with waxy wheat flour; (C) a cross-section of buckwheat noodles made with non-waxy wheat flour; (D) the surface of buckwheat noodles made from buckwheat flour alone; (E) the surface of buckwheat noodles made with waxy wheat flour; (F) the surface of buckwheat noodles made with non-waxy wheat flour.

remains obscure. In addition, there was a difference in the SEM between buckwheat noodles made with waxy wheat flour ((B) and (E) in Fig. 5) and buckwheat noodles with non-waxy wheat flour ((C) and (F) in Fig. 5). This difference in SEM may be ascribable to the presence of the amylose molecule in the non-waxy wheat flour, although the detailed information remains uncertain. On the other hand, buckwheat noodles made with waxy wheat flour ((B) and (E) in Fig. 5) had unique structural features together with a highly expanded structure. This unique structure of the buckwheat noodles made with waxy wheat flour may be closely associated with their observed mechanical characteristics (Figs. 1, 2 and 3).

The present study has demonstrated the mechanical

characteristics of buckwheat products made with the addition of waxy wheat flour. The results found suggest the possibility of development of buckwheat noodles made with the addition of waxy wheat flour as a new product.

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Characterization of graded buckwheat flours and some properties of germinated 'Mancan' buckwheat grains

Kazuyoshi MIYAKE[1], Rieko MORITA[1], Tri HANDOYO[1], Tomoko MAEDA[2] and Naofumi MORITA[1]*

[1] *Graduate School of Agriculture and Biological Sciences, Osaka Prefecture University, Sakai, Osaka 599-8531, Japan*
[2] *Department of Life and Health Sciences, Hyogo University of Teacher Education, 942-1, Shimokume, Yashiro, Hyogo 673-1494, Japan*

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Key words: graded buckwheat flours, mineral content, germination, gamma-amino butyric acid

ABSTRACT

The characteristics of 5 kinds of buckwheat flours [(special flour (FS), 1st–3rd grade flours (F1–F3) and hull flour (FBB)) obtained from milling and grading processes were determined. The fraction F3, which included the bran and the germ was the richest in nutritional components, showed the highest maltose value, and contained the largest amount of damaged starch among the tested samples. The germination speed of buckwheat was faster than that of wheat seeds. During germination, at 25°C, the amounts of reducing sugar and gamma-amino butyric acid (GABA) and the activities of α-amylase and protease increased more than those of wheat. Therefore, each flour fraction exhibited quite different characteristics depending on the portion of buckwheat they were derived from. The present milling and grading system for buckwheat is therefore very effective for practical applications. In addition, the flour from germinated buckwheat seeds were found to contain functional materials and therefore could provide for the production of new types of buckwheat-added foods with additional and functional properties.

INTRODUCTION

In general, the cereal grains contain various types of nutrient compounds including vitamins, minerals, and unsaturated fatty acids. However, we usually remove and discard most of these important compounds during the milling or polishing processes. If we could use the whole grain in our daily diet, it would not only be helpful in providing a better nutritional balance but also useful for us to be prepared for a future food crisis. Recently, brown rice without the hull was found to germinate normally. Germination has been considered to a convenient method to improve the functional and nutritional properties of rice, as the germination process increases the amounts of vitamins, minerals, fibers and physiologically activated materials as compared to non germinated rice (Kayahara, 2001). Actually, gamma-amino butyric acid (GABA), a functional substance, has been reported to increase in the germination process (Watanabe et al., 2004). In addition, during germination, the content of anti-nutritional materials such as trypsin inhibitors (Vidal-Valverde et al., 1994), tannins (El-Mahdy, 1985), pentosans and phytic acid are reduced (Udayasekhara, 1995).

Pre-germinated brown rice was the first of its kind that has become commercially available for our daily diet as a healthy ingredient. The amino acid score of buckwheat is much higher than that of rice or wheat, as buckwheat contains a large amount of lysine and tryptophan. Therefore, buckwheat grains were chosen for germination experiments with the expectation that their functional properties would increase. It was also expected that the allergenic substances of buckwheat would decrease during the germination process. The purpose of the present study was to determine the characteristics of various graded fractions obtained by milling the buckwheat cultivar Mancan and also to characterize the changes in functional properties of germinated buckwheat flour.

MATERIALS AND METHODS

Grain, flour and chemicals

The buckwheat grain used for germination was the cultivar 'Mancan' which had been imported from China and was obtained from Miyake Flour Milling Co., Ltd. (Osaka, Japan). The buckwheat grain was gradually milled from the innermost portion of grains; the five fractions, FS, F1, F2, F3 and FBB were obtained in the order of the innermost to the outermost fractions. The wheat grain used for germination was the hard-type wheat, No. 1 Canada Western Red Spring ('1CW'). All the other chemicals were of analytical grade without purification.

Basic analyses of flour contents

The water, starch, protein, lipid, dietary fiber and ash of all buckwheat fractions were determined following the procedures of AACC methods (AACC 44-15A; 46-11A;

* Corresponding author. TEL: +81-72-254-9459, FAX: +81-72-254-9921, E-mail: morita@biochem.osakafu-u.ac.jp

08-01 and 32-06, 2000). The lipid content was measured using an extraction method with *n*-butanol (Niihara, 1994) and the starch content was calculated as the remaining amount after subtraction of these components from 100.

Flour qualities of buckwheat fractions

The maltose value and the amount of damaged starch of the buckwheat flours were determined according to the procedures in the AACC methods (AACC 22-15; 76-30A, 2000). In addition, the mineral components were determined using a CCD Simultaneous ICP-OES apparatus (model Vista-Mpx AX3ch P/M, Seiko Instrument Inc., Tokyo, Japan) according to the manual as provided by the company. Ash from the flour samples (1 g) was prepared using a crucible (AACC 08-01, 2000), and the sample was then dissolved with 0.3 N HCl, and passed through a filter (0.45 μm). An aliquot of the filtered solution was used for the determination by an ICP apparatus.

Amino acid composition of flour fractions

Twenty mg each of the 5 fractions of the buckwheat flours were added to 1.5 ml of 6 N HCl and the proteins in the flours were decomposed to amino acids under a reduced pressure (100°C, 20 hr). For evaporation of the hydrolyzed solution, the HCl was evaporated to dryness using methanol. 5 ml of 0.02 N HCl was then added to the residue which was incubated during rotational mixing (40°C, 30 min), followed by centrifugation. The supernatant solution was then passed through a filter (0.45 μm), and an aliquot (50 μl) of the sample was analyzed using a LC-11A amino acid analyzer (Yanako, Kyoto, Japan). In the present study, hydrolysis with formic acid was not conducted for the determination of methionine and cystine.

Scanning electron microscopy (SEM) observation

SEM using a Hitachi apparatus (Hitachi Model S-800) was utilized to observe starch granules of the different fractions of flour using the procedures as described previously (Morita et al., 1996). The buckwheat fractions were stained over an OsO_4 solution for 12 hr, and the OsO_4 gas was completely removed under the reducing pressure over NaOH as a desiccant. The SEM samples were then placed on the sample stage and coated with Pt-Pd for 4 min. The SEM apparatus was operated at 10 kV.

Preparation of germinated grains

Whole buckwheat grains were soaked in water for 1 hr at 25°C, and then germinated for specified times under a relative humidity of 85% at 25°C. In contrast, wholewheat grains were soaked in water at 30°C for 5 hr. The water was then discarded through a mesh filter, followed by germination under a relative humidity of 85% at 30°C. As the buckwheat may become infected by bacteria during incubation at 30°C, the temperature was controlled to 25°C. After germination for 8, 16 and 24 hr, the buckwheat grains were collected, frozen, and then lyophilized. The lyophilized grains were pulverized to produce the powder samples using a milling apparatus. These grain flours were then used for the following experiments.

Effects of germination on the characteristics of buckwheat

Properties of germinated buckwheat

Basic analyses of the flour content of germinated buckwheat samples were conducted using the methods as described above. In addition, the effect of germination on the properties of the buckwheat flour obtained was determined from the amounts of total sugar, reducing sugar and damaged starch that were present using the procedures of phenol-sulfuric acid (Dubois, 1956), Somogyi-Nelson (Somogyi, 1945, 1951) and AACC methods (76-30A, 2000), respectively.

Mineral contents

Mineral contents of the powder samples of germinated buckwheat grains were determined using a fluorescent X-ray elemental analyzer MESA-500 (Horiba Instrument Co., Ltd., Kyoto, Japan) using a procedure as described previously (Maeda and Morita, 2000).

Enzyme activity of germinated buckwheat

Changes in α-amylase and protease activities in the germinated buckwheat samples were measured using a spectrophotometric method (Takahashi and Wada, 2002). Soluble starch and casein aqueous solutions were used as the substances for analyses of the amylase and protease activities, respectively. Iodine and Folin-Ciocalteu reagents were added to the reaction solutions for the amylase and protease activities, respectively and the absorbance of the color was measured at 660 nm. Amylase and protease activities of buckwheat during germination have previously been reported by Belozersky and Dunaevsky (1983), Dunaevsky et al. (1983), Kikunaga and Takahashi (1992, 1993). In addition, the inhibition of digestive enzymes has been also studied by Ikeda et al. (1984, 1991) and Tsybina et al. (2001, 2004). In the present study, the differences in enzyme activities between buckwheat and wheat grains after germination were evaluated by comparing the results of the present study with those mentioned above.

Amino acid of germinated buckwheat

Five g of germinated buckwheat powder from each of the different germination times were suspended in 30 ml of a 75% alcohol aqueous solution, and mixed vigorously with a vortex mixer at 80°C for 30 min. The suspension was then centrifuged at 2,100×g for 10 min. The same volume of 75% alcohol solution was again added to the precipitate, and the extraction was repeated twice using the same procedure as described above. The supernatant

that was obtained was pooled and evaporated to dryness under a constant temperature (35–40°C). 10 ml of 0.02 N HCl was added to the residue which was then centrifuged at 10,000 *g* for 10 min. The supernatant was increased to 25 ml using 0.02 N HCl, filtered through a 0.45 μm filter and an aliquot (50 μL) of the sample was analyzed by a LC-11A amino acid analyzer (Yanako, Kyoto, Japan).

IP6 amount of germinated buckwheat

The amount of phytic acid (IP6) in the various germinated flour samples was measured using a spectrophotometric method (Latta and Eskin, 1980). One gram of germinated buckwheat powder from each of the different germination times was added to 20 ml of 2.4% HCl aqueous solution, followed by mixing for 1 hr. After centrifugation of the mixture (10,000 rpm, 15 min), 5 ml of the supernatant obtained was diluted to 25 ml with distilled water. Five milliliter of the diluted solution was then passed through an anion exchange column (Dowex AG-1-X8 chloride form, 200–400 mesh). The column was washed with 15 ml of 0.1 N NaCl and the elutate was recovered. Then the inorganic phosphorus in the sample was eluted with 15 ml of 0.7 N NaCl and the elutate was pooled. Three milliliter of the solution was added to 1 ml of Wade reagent (0.03% $FeCl_3 \cdot 6H_2O$ and 0.3% sulfosalicylic acid in distilled water), and then the liberated phosphorus was immediately measured at 500 nm using a Shimadzu spectrophotometer model UV-160 A (Kyoto, Japan).

RESULTS

Characteristics of various buckwheat flour fractions

Basic analyses of flour contents

The moisture of buckwheat flours decreased following the order of FS, F1, F2, F3 and FBB (Table 1). The amounts of protein, lipid and ash increased from the inner fraction FS to the outer fraction F3. FBB was the outermost fraction; however the amounts of ash, protein and lipid it contained were lower than those of F3. It was found that the FBB fraction contained 84.8% dietary fiber, while the groats, from which the fractions FS, F1, F2 and F3 are derived, had only 3.05% (data not shown). Therefore, the carbohydrate value of FBB was assumed to be mostly from the dietary fiber with the value (83.4%) as shown in Table 1. As the fraction F3 included the bran and germ it was considered to be the richest in nutritional components among the fractions.

Table 1. Proximate analyses of various buckwheat fractions

Sample	Moisture	Ash	Protein	Lipid	Carbohydrate
			(%)*		
FS	14.4	0.2	3.8	2.6	79.0
F1	14.3	0.5	5.5	3.0	76.7
F2	12.4	3.1	23.7	5.9	55.0
F3	10.9	5.2	38.1	8.8	37.0
FBB	7.2	2.6	4.0	2.8	83.4

FS, F1, F2, F3 and FBB were gradually milled in the order of the innermost to the outermost fractions of a whole buckwheat grain. FS, special flour; F2–F3, 2nd–3rd grade flours; FBB, hull flour.

*, Values were calculated on a dry flour basis.

Flour qualities of various buckwheat fractions

Buckwheat flour obtained from the inner fraction had a distinct increased amount of damaged starch as compared with that from the outer fractions. Namely, the F3 flour fraction showed the highest maltose value and the largest amount of damaged starch among all the flour samples, while the FBB flour showed lower values than those of the F2 and F3 flour samples. This tendency was similar to the results of the proximate analyses as described above. In their mineral contents, FS, F1 and F2 were found to contain a small amount of Ca, whereas F3 and FBB had a larger amount (Table 2). All fractions contained a large amounts of Fe and S, and especially FBB exhibited the largest amount of S among all the fractions evaluated.

SEM images and appearances of various buckwheat flour fractions

SEM images of the various fractions from the buckwheat grains are shown in Fig. 1-1. Since FS and F1 exhibited a similar appearance of the grains, although, the image of F1 was not shown in Fig. 1-1. From these images, the size of the starch granules ranged from 5–7 μm and 1–3 μm for the large and small granules, respectively. In the FS of the inner fraction, the large starch granules were gathered together and formed a

Table 2. Mineral components of various graded buckwheat flours

(mg/100 g·dry flour)

Element	FS	F1	F2	F3	FBB
B	0.27	0.18	0.79	1.43	0.54
Ca	6.6	8.0	14.6	43.4	117.6
Cu	0.13	0.14	0.32	0.73	0.33
Fe	0.30	0.48	2.73	5.03	1.27
K	140.7	190.4	1085.5	2208.9	905.6
Mg	22.8	34.8	228.3	432.2	85.4
P	52.6	92.3	597.0	1129.0	66.7
Pd	0.04	0.04	0.04	0.04	0.05
Rb	0.95	1.08	3.97	7.60	1.68
S	1.08	0.89	0.35	0.59	2.40
Se	0.06	0.11	0.10	0.01	0.08
Sn	0.00	0.01	0.00	0.02	0.00
Zn	0.27	0.42	2.34	4.41	0.52

Abbreviations are the same as in Table 1.

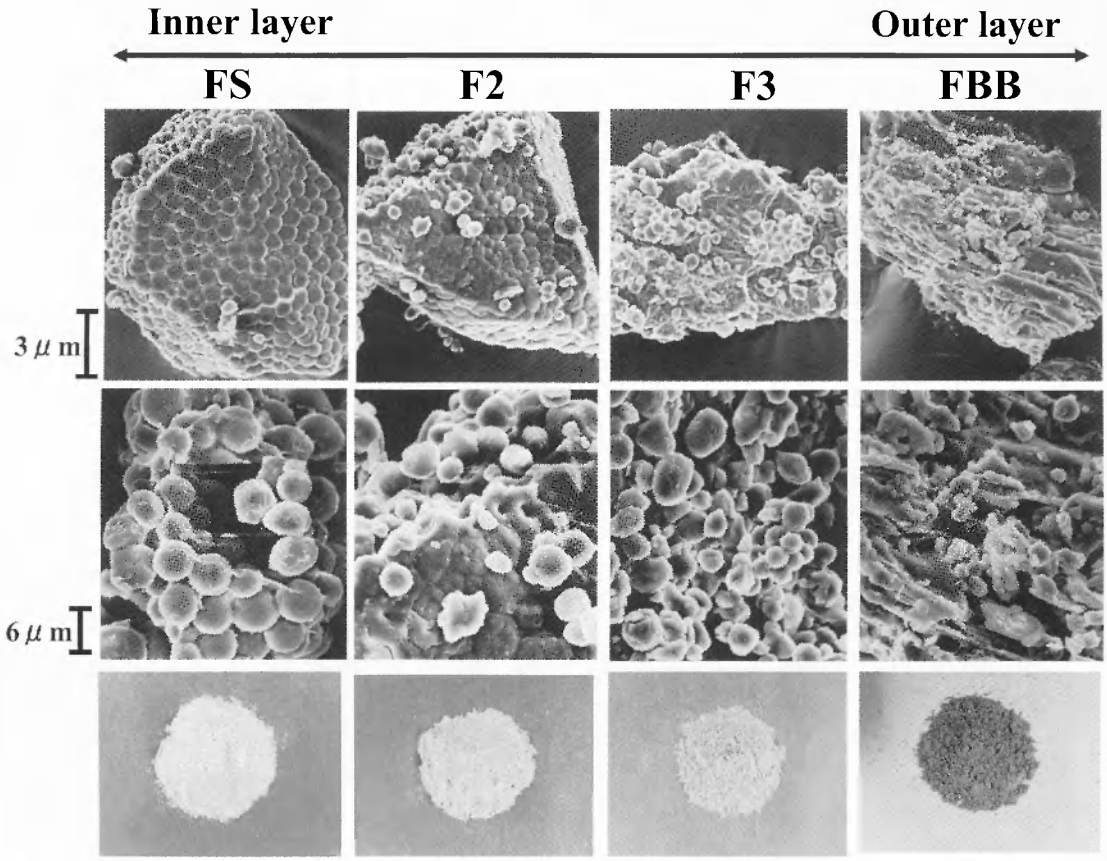

Fig. 1. SEM micrograph of various graded buckwheat grains and flours.
FS, F2, F3 and FBB were gradually milled in the order of the innermost to the outermost fractions of a whole buckwheat grain. FS, special flour; F2–F3, 2nd–3rd grade flours; FBB, hull flour.

large mass. In the F2 fraction, somewhat damaged starch granules were observed, and large amounts of fiber, attached to small starch granules, were observed. These flour samples containing fiber were considered to be derived from the seed coat or germ of the grain. Furthermore, the amount of starch granules decreased in the FBB, while the fibrous materials increased. The appearance of the buckwheat flours obtained from the various fractions were different among the samples and the color changed from brownish to whitish in the order of FBB, F3, F2, F1 and FS flours (Fig. 1-2).

Amino acid composition of buckwheat fractions

The amino acid compositions of the various buckwheat fractions are presented in Table 3. The F3 fraction, contained some germ and hull, had larger amounts of amino acids, as compared with the other fractions. The amount of GABA in the F3 fraction was clearly larger than those of the other samples. Although the FBB fraction was close to the F3 fraction in the relative amount of amino acid composition, the FBB had distinctly decreased amounts of amino acids when compared with F3. However, the amount of GABA in FBB was relatively larger when compared with the FS, F1 and F2 fractions.

Qualities of germinated buckwheat

Basic analyses of flour contents

After germination for 24 hr, the length of germs of buckwheat and wheat were approximately 2.0 and 0.5 mm, respectively. A longer and larger germ was observed in the buckwheat grains than in the wheat grains. The germination speed was obviously faster in buckwheat than in wheat. Before germination, the wheat grains of 1CW flour contained larger amounts of protein and lipid than was found in buckwheat (Table 4). After germination, the amount of protein in buckwheat slightly decreased, but there were no distinct differences in the other components of either wheat or buckwheat grain.

Carbohydrate properties of the germinated buckwheat

The amount of reducing sugar in the wheat and buckwheat grains increased during germination, and this phenomenon was clearer for buckwheat than for the wheat grains (Fig. 2). During the incubation for 16–24 hr, the largest amount of reducing sugar was produced in buckwheat.

IP6, enzyme activity and amino acid composition

The amount of IP6 in buckwheat was larger than that in the wheat grain before germination (Fig. 3). During germination, the value of IP6 in the buckwheat grain slightly

Table 3. Amino acid components in various graded buckwheat flours*

(mg/100 g·dry flour)

Amino acid	FS	F1	F2	F3	FBB
Asp	61	467	1603	2614	154
Thr	239	218	574	1052	150
Ser	302	312	991	1624	215
Asn	13	21	581	0	0
Glu	1535	1246	4964	8471	1461
Pro	195	99	1029	2133	189
Gly	646	567	1179	2027	333
Ala	505	300	900	1349	155
Val	301	33	887	1412	102
Cys	61	73	604	1016	52
Met	25	168	445	668	70
I-Leu	250	162	655	1199	1.9
Leu	454	374	1275	2181	196
Tyr	188	177	473	873	13
Phe	311	215	747	1211	90
GABA	9	3	87	310	89
Orn	128	20	66	15	1
Lys	500	370	1039	1800	224
His	184	767	362	547	28
Arg	641	419	2119	3870	91
Total amounts	7107	6531	21774	34954	4354

Abbreviations are the same as in Table 1.
*, All data were determined by hydrolysis with hydrochloric acid.

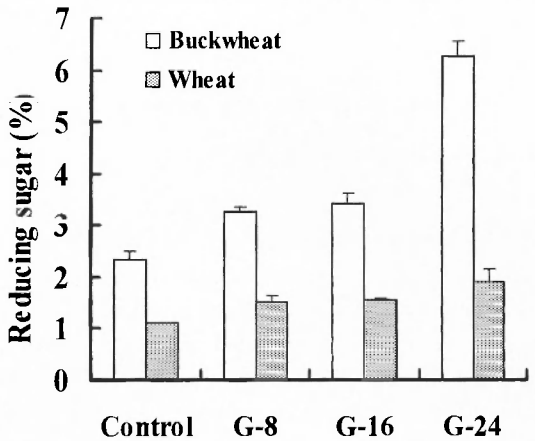

Fig. 2. Change of reducing sugar of buckwheat and wheat grains during germination.
Control, G-8, G-16 and G-24 are samples after germination for 0, 8, 16 and 24 hours, respectively. Values are calculated on a dry flour basis and vertical lines are standard deviation.

decreased, except at the 8 hr (G-8) incubation treatment, while that in wheat grain increased. However, after incubation for 24 hr, the amount of IP6 was found to be still larger in buckwheat than in wheat grain. Germination of

Table 4. Proximate analyses (mean±SD) of buckwheat (A) and wheat (B) grains after germination

(A)	Buckwheat	Ash	Protein	Lipid
		(%)*		
	Control	1.6±0.08	14.6+0.28	2.5+0.02
	G-8	1.7±0.03	13.5±0.30	2.3+0.04
	G-16	1.7±0.02	13.8±0.30	2.3+0.16
	G-24	1.7+0.02	13.8+0.10	2.3±0.10
(B)	Wheat	Ash	Protein	Lipid
		(%)*		
	Control	1.6+0.02	16.4+0.31	2.7+0.06
	G-8	1.6±0.01	16.2±0.34	2.7+0.15
	G-16	1.6±0.02	16.3+0.39	2.6±0.08
	G-24	1.6+0.01	16.3+0.28	2.6±0.19

Control, G-8, G-16 and G-24 are samples after germination for 0, 8, 16 and 24 hours, respectively.
*, Values were calculated on a dry flour basis.
SD, standard deviation.

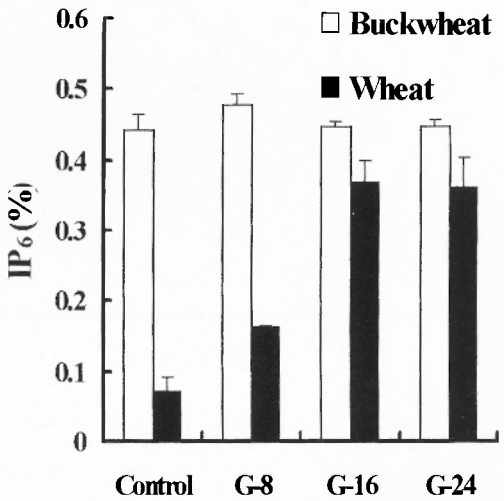

Fig. 3. Change of IP6 of buckwheat and wheat gains during germination.
Abbreviations are the same as in Fig. 2. Values were calculated on a dry flour basis and vertical lines are standard deviation.

buckwheat provided larger amounts of amino acid, especially GABA, where the amount after incubation for 24 hr increased 2.5 times compared to that before germination (Table 5). In contrast, the increased amount of GABA for wheat grain was 1.5 times under the same conditions. In addition, the value of lysine for buckwheat also increased during incubation as compared with that for wheat, with the total amino acid content in buckwheat being higher than that of wheat grain.

In enzyme activity, the total activities of α-amylase and protease of buckwheat grains were accelerated during germination, as compared with those of wheat grains

Table 5. Change of free amino acids of buckwheat and wheat grains during germination

	Buckwheat (mg/100 g·dry flour)				Wheat (mg/100 g·dry flour)			
Amino acid	Control	G-8	G-16	G-24	Control	G-8	G-16	G-24
THR	13.4	18.2	21.3	29.5	4.1	5.6	12.8	12.6
VAL	5.5	4.7	6.5	10.4	5.7	6.8	15.2	16.1
MET	8.8	9.9	10.3	13.2	2.1	5.9	5.1	5.3
LEU	11.7	13.2	13.0	16.9	3.8	7.2	16.8	15.9
TYR	8.7	11.2	9.9	10.7	4.0	3.6	11.5	12.5
PHE	10.2	11.9	13.2	16.8	3.3	4.8	11.6	12.0
HIS	5.6	10.6	13.7	23.2	2.7	4.0	9.2	8.5
LYS	9.6	10.4	13.2	19.6	9.2	10.4	12.8	17.6
GLU	72.4	75.4	105.6	133.4	36.7	59.6	94.1	82.6
GLN	16.5	27.3	44.6	88.4	13.7	5.0	34.5	34.6
GABA	12.4	11.6	22.5	28.7	5.2	3.6	6.9	7.9
Total	504.2	562.1	695.9	1011.3	373.4	421.0	575.9	564.3

Germinated samples are the same as in Table 4.

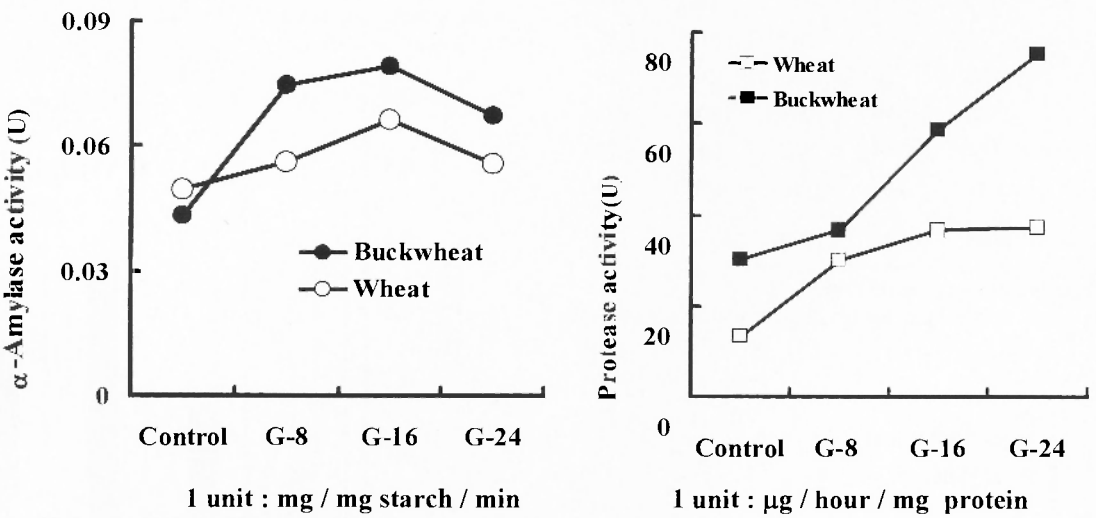

Fig. 4. Change of enzyme activities of buckwheat and wheat grains during germination. Abbreviations are the same as in Fig. 2.

(Figs. 4-1 and -2). After incubation for 16 hr, the α-amylase content of buckwheat showed a peak in activity, and the protease activity was found to increase with increasing length of incubation. This tendency was similar to previous reported results (Kikunaga and Takahashi, 1992, 1993; Belozersky and Dunaevsky, 1983).

DISCUSSION

From the present results, it was found that the nutritional or functional components of flour and the flour qualities are quite different among the different fractions obtained from the grains, from which the flour was milled. Therefore, the present grading process in the milling of buckwheat is very effective and important for the preparation of novel buckwheat flours. If the grading method is used for the preparation of buckwheat flours, various utilization of each fraction are possible which increase and extend the practical application of buckwheat. Furthermore, the various fractions prepared by grading the flour obtained from whole buckwheat grains may be effectively used to develop a study on patients allergic to buckwheat products. On the other hand, germination of buckwheat was considered to improve its functional properties, such as enzyme activity, amino acid and reducing sugar content and its treatment is thought to provide buckwheat with additional properties, which may result in the development of new processed foods. The germination process for buckwheat may be utilized for the improvement of the taste of the final products produced by the larger amounts of reducing sugar and free amino acids present, as compared with those in non ger-

minated buckwheat flour. Therefore, it is felt that combinations of germinated buckwheat and grading methods in the preparation of buckwheat flours may provide novel foodstuffs with high nutrition and with varied taste.

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Substitution of buckwheat for wheat flours on processing pasta and cookie

Tomoko MAEDA,[1] Kazuyoshi MIYAKE,[2] Madoka TAHARA[2] and Naofumi MORITA[2*]

[1] *Department of Life and Health Sciences, Hyogo University of Teacher Education, 942-1, Shimokume, Yashiro, Hyogo 673-1494, Japan*
[2] *Graduate School of Agriculture and Biological Sciences, Osaka Prefecture University, Sakai, Osaka 599-8531, Japan*

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ABSTRACT

Buckwheat flour obtained from milling groats and whole grains were used for the production of pasta and cookies. The light colored flour (LBW) which was composed of 100% groats, and the dark colored flour (DBW) which was composed of 81% groats plus 19% hulls, were used for quality evaluation of the flour and of the final products. The buckwheat dough absorbed a larger amount of water, and became weaker than dough made from American Western White wheat flour (AWW). An increase in the substitution of LBW or DBW for AWW increased water absorption. The addition of LBW (up to 30%) to common hard-type wheat flours (1CW) or durum wheat flours did not affect the tensile strength which remained at a value similar to that of the 1CW pasta sample. A sensory evaluation of the pasta made with DBW substitution of up to 30% with 1CW showed that the appearance, flavor and hardness were not poor as compared to those made from 1CW. The addition of LBW (up to 30%) to AWW increased the whiteness of the cookies, while DBW decreased it. Substitution of LBW or DBW for AWW suppressed the spread of the cookie paste during baking and decreased the final firmness of the baked cookies. The application of DBW which contains the hull material was expected to add new taste and rheological properties to the final processed products.

INTRODUCTION

The addition of various additives and the substitution of new ingredients in the production of breads and noodles have been used to improve dough and product properties, nutritional value and cost performance. Buckwheat is one of the traditional food products for Japanese, however the utilization of buckwheat in processed foods is very limited. It has mainly been used for the production of Japanese-style *soba* or buckwheat noodles. However, recently the high levels of the functional properties in buckwheat have increased focus on this crop. Buckwheat protein contains higher levels of some essential amino acids, such as lysine, threonine and tryptophan (Nair and Adachi, 1999), as compared to the cereals. Furthermore, buckwheat is rich in minerals, vitamins, flavonoid rutin and catechins (Oomah and Mazza, 1996; Watanabe, 1998; Steadman et al., 2000, 2001a). Buckwheat bran especially includes high concentrations of protein 36%, lipid 11%, dietary fiber 15% and minerals 7% (Steadman et al., 2001b). The bran also contains fagopyritols, a galactosyl derivative of D-*chiro*-inositol that may be useful in the treatment of non-insulin-related diabetes mellitus (Ostlund et al., 1993). Therefore, the characteristics of the starch, protein and lipids in buckwheat have been studied to evaluate their nutritional potential and application as a new ingredient in the area of cereal science (Li et al., 1997; Qian et al., 1998; Tsuzuki et al., 1991; Mazza, 1998; Skerritt, 1986). In addition, the characteristics of buckwheat flours during milling or extrusion processes have been evaluated to assess the optimum conditions for extrusion and to obtain potential dietetic flours from the milling by-products (Rayas et al., 1998; Mazza and Campbell, 1985; Skarabanja et al., 2004). The medicinal effects of buckwheat for clinical applications have been widely investigated (Lu et al., 1992; Song, 1992). In contrast, although the addition of buckwheat for the production of processed foods and for the properties of noodles containing buckwheat, its bran and its protein have been reported (Manthey et al., 2004; Bejosano and Corke, 1998; Rayas-Duarte et al., 1996), the studies are very few, as compared with those of wheat flours. Only limited information is available concerning the effects of buckwheat flours on the qualities of some products, such as cookies or noodles.

The purpose of the present study was to determine the optimum amount of buckwheat flour which could be added for the production of wheat noodles or cookies and to determine its possible utilization in these products in order to improve the functionality of the final products, including quality parameters such as taste, texture and nutrition.

*Corresponding author. TEL: +81-72-254-9459, FAX: +81-72-254-9921, E-mail: morita@biochem.osakafu-u.ac.jp

MATERIALS AND METHODS

Flour and chemicals

The wheat flour used included a commercial hard-type wheat flour; No. 1 Canada Western Red Spring (1CW), a soft-type wheat flour; American Western White (AWW) and durum wheat flour obtained from Miyake Flour Milling Co., Ltd. (Osaka, Japan). Two types of buckwheat flour were prepared. One from the groats, light color buckwheat (LBW), which was composed of 100% groats flour and another from the hull of the variety Mancan provided from the Miyake Flour Milling Co., Ltd. The two flours, with a particle size of 100–150 μm, were mixed together to achieve the same ratio as is found in the original buckwheat grain. This was called dark color buckwheat (DBW), and was composed of 81% groats and 19% hull flours. The total amounts of dietary fiber for the groats and hull flours were 3.1 and 84.8%, respectively, as evaluated by the enzyme-weight procedure of the Prosky method.

Flour qualities

The mixing properties of the various flours were determined from Farinograph mixing. The Farinograph mixing was carried out using a Brabender apparatus equipped with a 50 g stainless steel bowl, and the mixing was operated at a standard speed of 63 rpm at 30°C (AACC 54-21, 2000). In addition, the color of the various flours was determined from the values of lightness or hue by a Color Reader CR-13 (Minolta, Tokyo, Japan).

Cookie making

Zero to 50% of AWW was substituted for by either LBW or DBW in the production of cookie samples made according to the formula and procedures of the AACC method (10-50D, 2000) with minor modifications.

Preparation of various pasta samples

The pasta samples were made using 500 g of combined buckwheat and wheat flour, 170 g of water and 10 g of salt according to the AACC method (66-50, 2000). Zero, 30, 40 and 50% of 1CW or durum wheat flour were substituted by LBW, and raw, dried and cooked pasta samples were prepared. The cooked pasta samples were prepared by boiling (2.5 min) and the raw pasta without drying. In addition, cooked pasta samples were also prepared by boiling the dried pasta (40°C, 18 h) for 4.5 min or the optimum time. In total, four types of pasta samples were used for evaluation. The optimum boiling time for each pasta sample was decided by adjusting to the same amount of swelling of the boiled pasta among all the samples.

Rheological properties of pasta and cookie

The rheological properties of the cooked pasta samples and the cookies were measured by the same Rheoner RE-3305 (Yamaden Co., Ltd., Tokyo, Japan) as reported previously (Morita et al., 2002).

Appearance of cookie and pasta samples

The effects of LBW or DBW substitution for wheat flour in the cookie and pasta samples were examined from the appearance and color. The values of lightness and hue of the samples were measured by the same Color Reader CR-13, as described above.

Sensory test of pasta sample with buckwheat and hull

In an effort to evaluate the use of buckwheat as a practical application, the pasta samples made from DBW by 30–50% substitution of the wheat flour, using a recipe for commercial pasta were tested by sensory evaluation. The prepared pasta samples were evaluated by twenty panelists including teachers and students in the College of Agriculture of Osaka Prefecture University. Ten characteristics including, color, appearance, flavor, hardness, elasticity, viscosity, smoothness, feeling on the tongue, taste, evaluation for the quality of the pasta samples were measured using a scoring method with 11 levels.

The experimental tests as described above were repeated at least three times.

RESULTS

Characteristics of buckwheat flours

Farinograph mixing

The water absorption of the AWW, light buckwheat flour, 1CW and durum flours were 53.4, 60.4, 66.0 and

Table 1. Farinograph data of various flours

Sample	Water absorption (%)	Development time (min)	Stability time (min)
AWW	53.4	1.3	6.0
LBW	60.4	1.0	0.5
DBW	60.4	1.0	0.5
1CW	66.0	18.5	26.0
Durum	62.6	3.0	4.5
L-10	53.8	0.6	4.1
L-20	54.2	0.7	3.2
L-30	54.3	0.8	4.0
L-40	54.5	0.7	4.5
L-50	54.8	0.8	0.4
D-10	54.8	0.9	3.9
D-20	55.4	1.5	4.9
D-30	56.0	0.8	1.0
D-40	58.0	4.5	9.9
D-50	58.6	5.0	5.8

1DW, commercial hard-type wheat flour; Durum, commercial durum flour; AWW, commercial soft-type wheat flour; LBW, light-color buckwheat flour composed of 100% groats flour; DBW, dark-color buckwheat flour composed of 81% groats and 19% hull flours. N=3.

L-10–L-50 and D-10–L-50 are 10–50% substituted AWW flours with LBW and DBW, respectively.

62.6%, respectively (Table 1). The water absorption of the hard-type wheat flour of 1CW was very stable, as compared with the other flours. The buckwheat flour absorbed a larger amount of water than did the soft-type AWW, and the Farinogram of the LBW was weaker than that of AWW. As the amounts of LBW or DBW substitution for AWW increased, the optimum amount of water absorption also increased accordingly. The addition of DBW, which containing hull material, to AWW distinctly increased the water absorption. In addition, it was found that the substitution of LBW or DBW resulted in two peaks in the Farinogram, and the peaks became quite prominent at 50% LBW or DBW substitution.

Color of flours

The values of whiteness or lightness of the flours decreased in the order of AWW, 1CW, LBW, durum and DBW flours (Table 2). The hue value of b^* for durum wheat flour was the highest among all the flour samples. The a^* and b^* values for LBW flour were similar to those of 1CW, but the DBW flour was reddish and dark in color.

Characteristics of processed foods containing buckwheat flours

Color of cookie samples containing buckwheat and hull

Cookie samples containing LBW or DBW are shown in Fig. 1. As the LBW substitution for AWW increased, the color of the cookie samples became increasingly white. However, DBW substitution decreased the whiteness and lightness with increasing amounts of DBW (Table 3). Therefore, the addition of either LBW or DBW resulted in different colored cookie samples.

Rheological properties of cookies baked

The increase in the amount of LBW or DBW substitution for AWW decreased the firmness of the baked cookies, as compared with those made from AWW alone. The substitution of LBW resulted in increased softening of the cookies with an increasing amount of substitution, while the substitution of DBW resulted in a similar firmness, regardless of the amount of substitution. The addition of both LBW and DBW lowered the values of cookie spread during baking, depending on the amounts of additives, when compared with the AWW cookie sample. This tendency was more obvious for DBW than for LBW. DBW suppressed the spread of the cookie paste during baking, as compared with the AWW sample.

Characteristics of various pasta samples

The addition of LBW (up to 30%) to the 1CW or durum wheat flours did not affect the tensile strength, which kept a value similar to that of the 1CW pasta sam-

Table 2. Summary of color properties of various flours

Sample	L^*	a^*	b^*	Whiteness
AWW	89.3	0.3	8.8	73.2
LBW	82.9	0.9	10.4	62.9
DBW	71.3	1.2	6.4	48.8
Hull	47.6	4.7	9.8	20.6
1CW	87.7	0.8	10.3	69.5
Durum	81.8	1.5	22.6	49.3

Sample	L^*	a^*	b^*	Whiteness
AWW (wet)	82.6	−1.3	9.6	63.1
LBW (wet)	73.5	0.1	8.2	51.2
DBW (wet)	48.6	2.6	4.6	22.3
Hull (wet)	24.9	3.7	3.2	5.1
1CW (wet)	80.0	0.4	12.1	57.7
Durum (wet)	76.7	0.6	19.6	47.4

Abbreviations are the same as in Table 1, except that hull is flour obtained from hull of buckwheat.

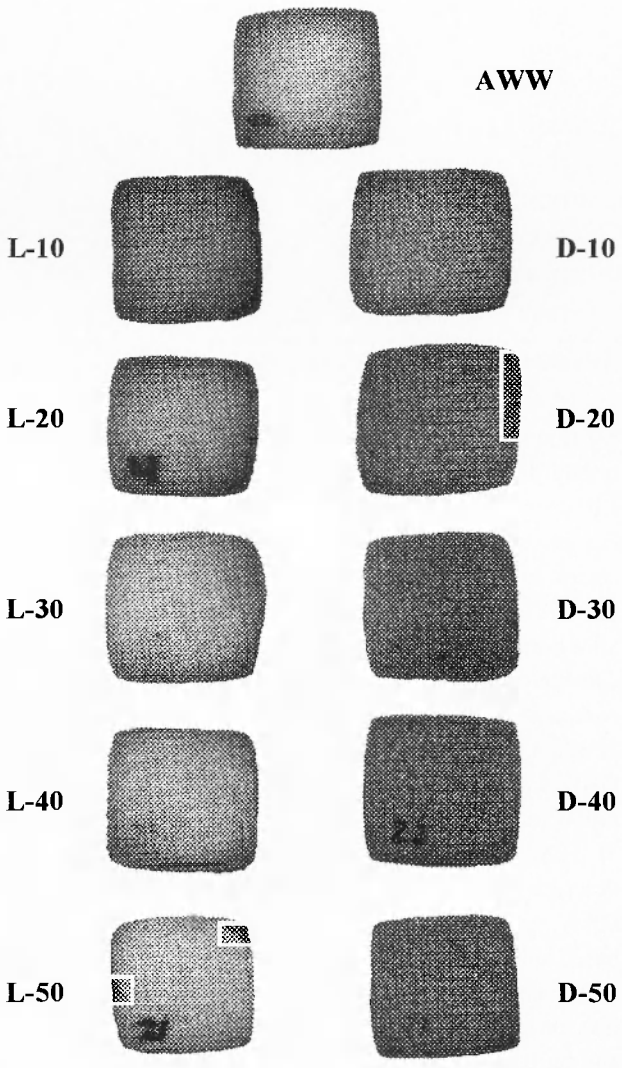

Fig. 1. Appearance of cookie samples containing buckwheat flours. Abbreviations are the same as in Table 1.

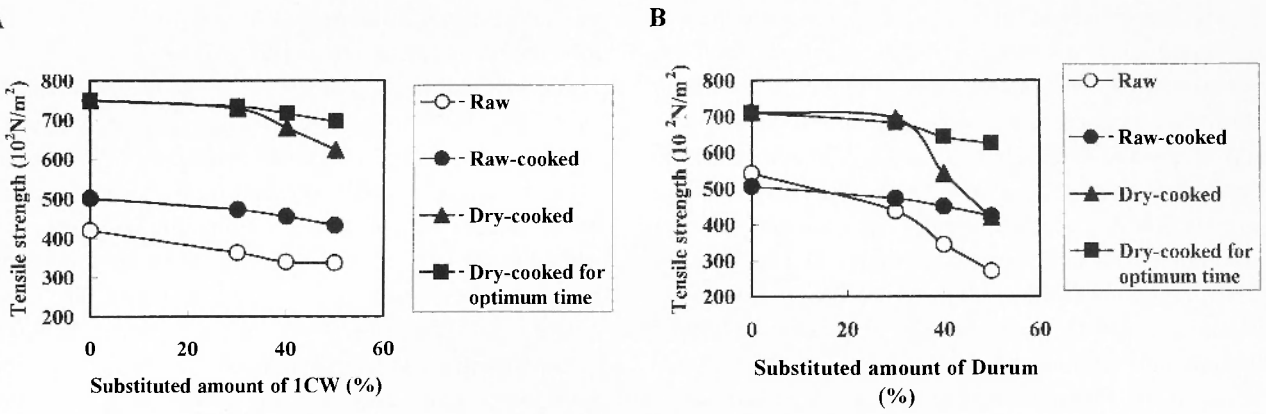

Fig. 2. Effects of buckwheat flours on the tensile strength of pasta samples. 1CW or durum flours were substituted with LBW.

Table 3. Summary of color properties of cookies baked with buckwheat flours

Sample	L*	a*	b*	Whiteness
AWW	69.0	5.0	25.0	35.0
L-10	70.5	5.4	25.7	35.6
L-20	69.2	5.1	24.4	35.5
L-30	72.8	2.5	21.8	41.3
L-40	69.7	4.3	23.5	36.7
L-50	70.5	4.4	23.9	37.2
D-10	61.7	3.5	15.4	33.4
D-20	55.2	4.0	13.4	27.2
D-30	54.0	3.4	11.3	26.6
D-40	51.5	3.6	10.7	24.2
D-50	49.2	3.7	10.1	22.1

Abbreviations are the same as in Table 1.

ple (Fig. 2). But, a 50% substitution for durum flours distinctly decreased the strength. The lowering tendency of LBW on the tensile strength was more pronounced for the substitution of the durum flours than that for the 1CW flours. In addition, LBW substitution for 1CW or durum flours did not decrease the whiteness of pasta samples after boiling (Table 4).

Characteristics of pasta samples made by the recipe of commercial pasta

When the pasta samples were made from 1CW and DBW following a commercial recipe, 30% DBW substitution for 1CW showed a similar tensile strength to that of 1CW alone. This tendency was the same as that described above. Furthermore, up to 30% substitution of DBW, as a practical application of DBW may be possible based on the results of the sensory test. Namely, when 1CW was substituted with 30% DBW, there were no significant differences ($p<0.05$) on the appearance, flavor and hardness factors between the 1CW and 30% DBW-substituted samples.

Table 4. Summary of color properties of various pasta samples containing buckwheat flours

A

Sample	L*	a*	b*	Whiteness
Durum	76.6	0.9	11.1	53.89
Durum-30	71.4	1.3	12.8	46.11
Durum-40	71.2	1.4	11.2	46.70
Durum-50	70.1	1.7	11.9	44.93
1CW	79.4	0.4	10.5	58.10
1CW-30	75.8	0.7	10.7	53.07
1CW-40	74.4	0.9	10.4	51.35
1CW-50	72.5	1.1	10.0	49.01

B

Sample	L*	a*	b*	Whiteness
Durum	82.6	1.1	16.6	56.6
Durum-30	77.6	1.6	17.9	49.8
Durum-40	79.4	1.4	15.7	53.9
Durum-50	80.9	1.0	12.7	58.4
1CW	82.4	1.4	14.3	58.7
1CW-30	79.9	1.4	13.3	56.5
1CW-40	79.2	1.5	13.9	55.2
1CW-50	81.9	1.0	11.2	60.9

C

Sample	L*	a*	b*	Whiteness
Durum	66.6	−0.7	9.1	41.7
Durum-30	63.9	0.8	9.3	38.3
Durum-40	65.2	0.5	8.1	40.3
Durum-50	63.4	1.1	8.4	38.0
1CW	67.1	−2.0	4.9	43.5
1CW-30	67.3	−0.4	6.4	43.5
1CW-40	66.5	0.3	6.5	42.4
1CW-50	65.8	0.9	6.7	41.4

A, B and C are results of raw, raw-cooked and dry-cooked pasta samples, respectively. Abbreviations are the same as in Table 1, except that Durum-30–50 and 1CW-30–50 were 30–50% substituted Durum and 1CW flours by LBW, respectively.

DISCUSSION

The present results indicated that the flour quality of buckwheat was relatively low as determined from the farinograph mixing data. In processed foods, the substitution of LBW or DBW for 1CW did not affect the tensile strength, with it keeping a similar value to that of the 1CW pasta sample. As a 30% substitution of DBW for common wheat flour in the production of pasta using a commercial recipe did not significantly ($p<0.05$) decrease the rheological properties of the normal pasta sample without any DBW substitution from the results of a sensory test, pasta products containing hull could be accepted as daily commodities in the near future. Furthermore, since various types of pasta are sold in markets in Japan, the pasta made from substitution by DBW, which contains some hull materials, could be accepted by the consumers. As for the cookies containing buckwheat, substitution of either LBW or DBW, did not increase the hardness of cookies, and the spread of the cookie paste during baking was suppressed. Furthermore, the appearance seemed to be quite stable, as compared with AWW. The substitution of LBW was found to not decrease the whiteness of cookie sample. Therefore, the addition of DBW buckwheat flour can be expected to be utilized for the production of processed foods to add or change their functional properties, although the bran and hull are usually discarded as by-products after milling buckwheat.

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Short Communication

Evaluation of seed-dressing fungicides against sclerotinia root rot of buckwheat

Kalyan Kumar MONDAL

Mountain Agricultural Research & Extension Centre, CSK Himachal Pradesh Agricultural University, Sangla, Kinnaur 172 106, Himachal Pradesh, India

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Key words: buckwheat, disease, sclerotinia root rot, fungicides

ABSTRACT

Buckwheat (*Fagopyrum esculentum* Moench and *F. tataricum* Gaertn.) is produced extensively in the temperate regions of India, contributing to the regional economies. Sclerotinia root rot incited by *Sclerotinia sclerotiorum* (Lib.) de Bary is one of the major threats to the buckwheat seedlings, creating poor stands of the crops. To date there are no fungicidal recommendations available for this dreaded menace for the buckwheat growers of these regions. Therefore, the present study grown in pots in a glasshouse, concentrated on screening seed-treatment fungicides for effective management of buckwheat root rot. Among the fungicides tested, ridomil MZ 72 WP (metalaxyl 8%+mancozeb 64% WP) which is mixture of systemic and contact fungicides, was found to give the best protection against root rot incidence in both common and Tartary buckwheat (by 50 and 56% protection of root rot incidence over the control, respectively) and thereby increased the plant stand (by 45 and 62% in common and Tartary buckwheat, respectively) over the untreated control plants.

INTRODUCTION

Buckwheat is one of the more important crops cultivated extensively in the temperate regions of India, especially in the western parts of the Shimla, Kinnaur and Lahaul districts of Himachal Pradesh (Joshi, 1999; Joshi and Paroda,1991; Mondal et al., 2002a, b, 2003a). Buckwheat flour is often blended with wheat flour for the preparation of "chillare", a popular dish of these regions (Singh and Thomas, 1978; Mondal et al., 2003a). A serious biotic threat limiting the productivity of buckwheat is root rot, induced by *Sclerotinia sclerotiorum* (Mondal et al., 2002a), which is the most alarming as it causes more than 35 per cent seedling mortality (Mondal et al., 2002a, 2003b). This disease has been reported to be more severe on Tartary buckwheat (*F. tataricum* Gaertn.) resulting a poor stand of seedlings (Mondal et al., 2002a, 2003b). The disease symptoms appear as water soaked areas which soon turn into brown patches, on the upper portion of the root. The patch gradually proceeds downward to cover the total root system. The above ground foliage wilts and dies either gradually or quickly. It spreads rapidly under cloudy and humid weather conditions with day time temperatures ranging from 15–20°C (Mondal et al., 2003b). In view of the devastating nature of the disease, the present fungicidal evaluation program was initiated under glasshouse conditions in an attempt to determine the most effective seed-dressing chemical which could be utilized in suppressing this root rot pathogen.

MATERIALS AND METHODS

The fungicidal screening program was conducted as a pot study in a glasshouse. Seeds of both the species (common and Tartary buckwheat) were treated with three different fungicides, namely bavistin (carbendazim 50 WP) 0.1%, raxil (tebuconazole 2%) 0.1% and ridomil MZ 72 WP (metalaxyl 8%+mancozeb 64% WP) 0.25%. For the fungicides that required a wet dressing (as in the case of bavistin and ridomil) the seeds were soaked in a fungicidal solution in water (1 g per litre of water) for 30 minutes and then dried in a shed on a blotting sheet for 1 h. The dry seed dressing raxil was performed by thoroughly mixing the seeds with the fungicide (@1 g/kg seed) inside a container before sowing. Plastic pots (10 inch×8 inch) were filled with pre-autoclaved (at 121.6°C for 20 minutes at 15 lb pressure for consecutive 3 days) garden soil comprised of sandy-loam soil and dried farmyard manure at a 3:1 ratio. The seeds were then sown at a depth of 2–3 cm. Mature sclerotium (4–5 mm diameter), which were harvested from 10–12 day old cultures of *S. sclerotiorum* grown at 30°C on a potato dextrose agar, were used as the inoculum. Immediately after sowing the seeds, the pots were inoculated with the sclerotia. The sclerotia (2–3 Nos per seed) were placed at the same point and depth, as the seeds to ensure that the germinating hyphae from the sclerotium could easily invade the vicinity of the seeds (to establish the initial infection). 25 treated seeds were sown in each pot and

there were 10 pots per treatment. Pots sown with untreated seeds and also inoculated with sclerotia were grown as the control. The seedlings were watered at regular intervals. Observations were recorded on 30 days old seedling on final seedling stand as well as on disease severity by following a 0–5 scoring scale (Mondal et al., 2003b). The percent protection of root rot incidence over the control as well as the percentage increase in plant stand over the control was calculated by using the following formula:

Root rot protection over control (%)=[(Disease score in treated pots–Disease score in control pots)/Disease score in control pots]×100

Increase in plant stand over control (%)=[(No. of healthy seedlings in treated pots–No. of healthy seedlings in control pots)/No. of healthy seedlings in control pots]×100

RESULTS AND DISCUSSION

In the present study, all the seed dressing chemicals showed significant protection against root rot (significant at 0.01 level by the test for the comparison of proportion of root rot over the control, see e.g. Snedecor and Cochran, 1967). Ridomil MZ 72 WP 0.25% gave the sig-

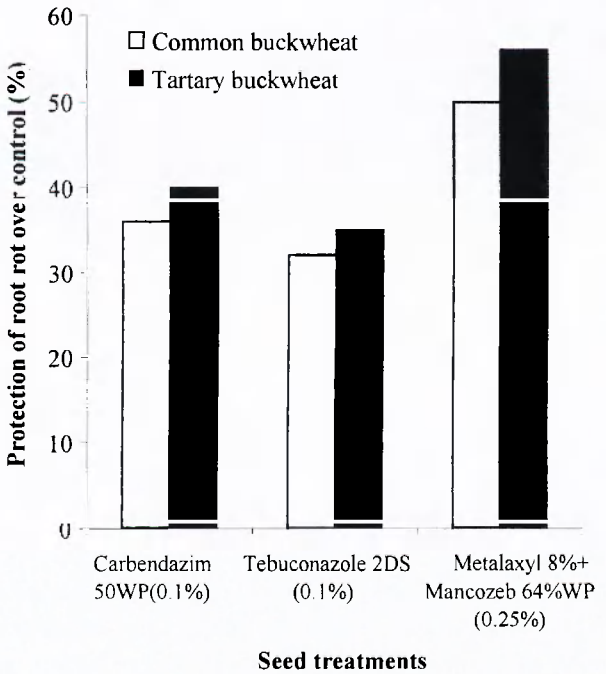

Fig. 1. Percent protection of sclerotinia root rot incidence on buckwheat over control as affected by different seed treatments.

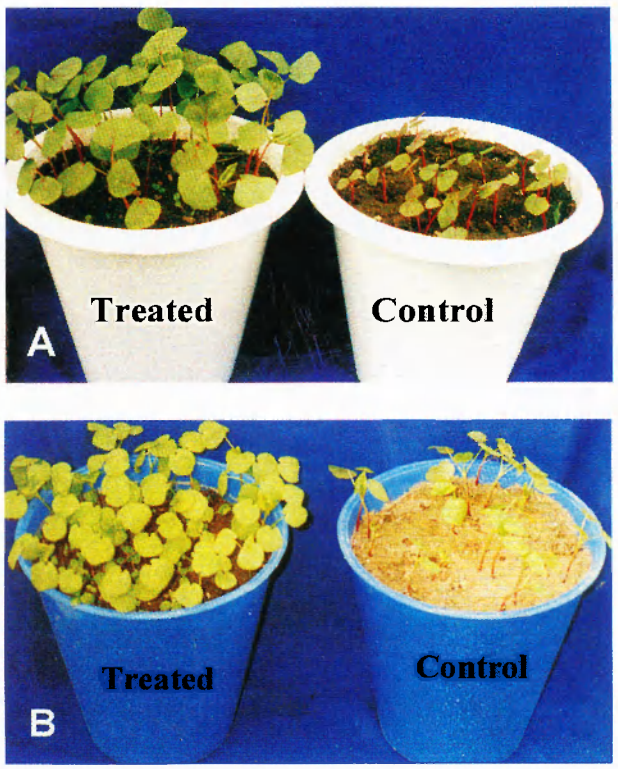

Fig. 3. Photographs showing good seedlings stand of tartary buckwheat (A) and common buckwheat (B) developed from treated (with ridomil MZ 72 WP 0.25%) seed (left pots) against the control seedlings as affected by sclerotinia root rot raised from untreated seed (right pots).

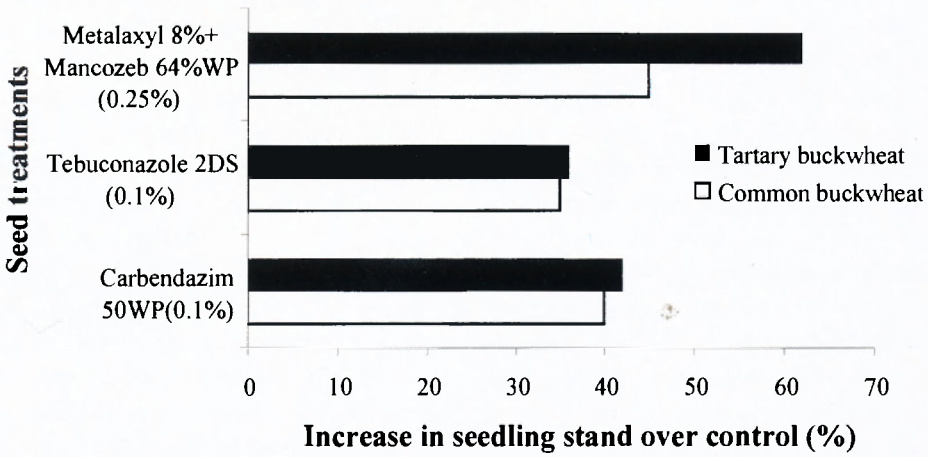

Fig. 2. Per cent increase in seedling stand of buckwheat over control as affected by different seed treatments used against root rot pathogen.

nificantly highest protection (Fig. 1), among the seed-dressing chemicals tested against root rot for both species of buckwheat (by 50% and 56% in common and Tartary buckwheat, respectively). It significantly increased (by 45 and 62% in common and Tartary buckwheat, respectively) the plant stand (Fig. 2 and 3) over the untreated control plants. The results also indicated that Tartary buckwheat, which has been reported to be more susceptible to root rot (Mondal et al., 2002a, 2003b) responded better than common buckwheat to the seed dressing treatments. This suggests that the seed treatment with ridomil MZ 72 WP could be more effective under even better conditions for compatible host-pathogen interactions. Fungicidal seed dressings for the management of root rot diseases have been well documented under varied host pathogen systems (Singh, 1998); however the fungicidal efficacy had not yet been elucidated for the buckwheat-*Sclerotinia* system, particularly for buckwheat growers in the temperate regions of India.

The superior performance of the fungicide, ridomoil MZ 72 WP, which is a combination of systemic (metalaxyl 8%) and contact fungicides (mancozeb 64% WP) in suppressing the root rot incidence may be explained by its two way action, i.e. quick absorption and subsequent translocation (in an acropetal direction in the transpiration stream) along the plant distribution system by the systemic activity of metalaxyl 8% as well as by the contact action of mancozeb 64% WP (Nene and Thapliyal, 1993). The development of resistant strains by fungal pathogens against metalaxyl (owing to be very selective in action) has been found to be a common phenomenon (Reuveni and Cohen, 1980); however, when it is used in combination with other fungicides, particularly with dithiocarbamates (such as mancozeb) the selection and build-up of metalaxyl-resistant fungal strains may be delayed and at the same time the spectrum of activity may be broadened (Nene and Thapliyal, 1993). Therefore, the present study was unique in identifying a suitable fungicide which would not only be effective in suppressing the dreaded root rot pathogen of buckwheat but would also be desirable in regards to delayed development of resistance by the causal fungus.

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Short Communication

Integrated weed management in buckwheat

Surendra Singh RANA, Pankaj SOOD and Man Chand RANA

Mountain Agricultural Research and Extension Centre, CSK Himachal Pradesh Agricultural University, Sangla, Kinnaur 172 106, Himachal Pradesh, India

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Key words: Weed, herbicide, economic threshold, weed index, weed control efficiency

ABSTRACT

Field experiments were carried out at Mountain Agricultural Research and Extension Centre Sangla during the summer seasons of 2001 and 2002, to evaluate some herbicides alone and in integration with hand weeding. Three herbicides *viz.* alachlor 1.50 kg/ha, oxyfluorfen 0.20 kg/ha and pretilachlor at 1.00 kg/ha, alone and at half rates supplemented with one hand weeding were compared to hand weeding and weedy check. Alachlor 1.50 kg/ha, alachlor 0.75 kg/ha + hand weeding, oxyfluorfen 0.10 kg/ha + hand weeding and pretilachlor 0.50 kg/ha + hand weeding gave satisfactory weed control and seed yields of Tartary and common buckwheat. *Digitaria sanguinalis* was the most competitive weed species. Alachlor 0.75 kg/ha + hand weeding resulted in minimum weed index (–12.0) in Tartary buckwheat and alachlor 1.50 kg/ha in common buckwheat (–41.7). Alachlor at 1.50 kg/ha resulted in maximum marginal benefit cost ratio of 6.72 in Tartary buckwheat and 9.37 in common buckwheat. Seed yields of Tartary and common buckwheat were negatively associated with weed count and weed biomass. The economic threshold of weeds at the prevalent price of treatments and crop production varied between 12.6–40.8 weeds per m^2 in Tartary buckwheat and 6.6–37.4 in common buckwheat.

INTRODUCTION

Buckwheat (*Fagopyrum tataricum* Gaertn. and *Fagopyrum esculentum* Moench) is the most important crop in the higher Himalayas, where summer is the only growing season. It is the only food crop that can be grown in quick succession after pea (Rana et al., 2003a), barley or wheat. It was regarded as a crop of poor soils (Anonymous, 1958), but the recent developments that the buckwheat has its own potential for fixing atmospheric nitrogen (Lakhova, 1998; Alekseyeva, 2002) will open up new vistas in the development of sustainable production systems in lower elevations as well. There is ample scope for buckwheat to be widely utilized as a green manure crop to reclaim degraded land.

Competition from weeds is the major production constraint impairing buckwheat productivity (Rana et al., 2003). Therefore, all weeds must be controlled (Parodi and Nebreda, 1998). Higher seeding rates, 2–2.5 times the normal rate used in the dry temperate zone, are generally used to smother weeds (Hore and Rathi, 2002; Chernetski, 1975). As well, one or two hand weedings are usually done to tackle the weed menace. Manual weeding not only takes a major share of the farm labour but is tedious and uneconomical. Herbicidal weed control has been found quite effective in buckwheat (Rana et al., 2003). Herbicides are effective to control initial flush of weeds which usually remain out of reach of the other methods of weed control. Weeds that can not be controlled by non-chemical methods can easily be controlled with herbicides. However, herbicidal weed control is not aimed at replacing any other non-chemical option available to the farmer. Rather, herbicides should be looked upon as supplement to cultural, physical and other methods of weed control to obtain superior and more efficient and economical control of weeds than is possible with the existing methods alone. Thus, the investigation was undertaken to evaluate the performance of herbicides applied alone and in integration with hand weeding.

MATERIALS AND METHODS

Field experiments were conducted during the summer seasons of 2001 and 2002 on sandy loam soils at Sangla (2591 m above msl) in randomized block design with three replications. Three herbicides *viz.* alachlor, pretilachlor and oxyfluorfen established selective earlier (Rana et al., 2003), at 1.50, 1.00 and 0.20 kg/ha, respectively were evaluated alone and at half rates along with hand weeding (30–35 DAS, days after sowing) and compared to hand weeding (30–35 DAS) and weedy check (Tables 1 and 3) in common and Tartary buckwheat. The Tartary buckwheat crop was sown in the last week of May and common buckwheat in the third week of June in the respective years. Tartary buckwheat genotype, Sangla B1 and common buckwheat, OC-2 was sown at 40 kg/ha in lines 30 cm apart. The seeding was done after pre-sowing irrigation and thereafter no water was applied. One

half of N (40 kg/ha), all P_2O_5 (40 kg/ha) and K_2O (20 kg/ha) was applied as basal. Remaining N was top dressed at 35 DAS. Herbicides were applied one day after sowing. Other management practices were in accordance with the package of practices for the crops (HPKV, 1993). Weed counts were made before the harvest of the crops at two random places using quadrat of 50 cm×50 cm. Dry weight of weeds was recorded after oven drying at 70±1°C for 72 h. Economics of treatments was computed based upon prevalent market prices. Weed control efficiency (WCE) and weed index (WI) were worked as per the formulae given below,

WCE (%)=[(Dry weed weight in weedy–Dry weed weight in treated plot)/Dry weed weight in weedy]×100

WI (%)=[(Yield from hand weeded plot–Yield from treatment plot)/Yield from hand weeded plot]×100

The weed index for treatments was worked out based upon hand weeding treatment.

Economic thresholds (=Economic injury levels) were determined by the method of Stone and Pedigo (1972) as below,

Economic threshold=Gain threshold/Regression coefficient

where, Gain threshold=Cost of weed control/price of produce, and Regression coefficient (b) is the outcome of simple linear relationship between yield (Y) and weed biomass (X), Y=a+bx.

RESULTS AND DISCUSSION

The major weed species infesting the Tartary and common buckwheat crops were, *Digitaria sanguinalis* L. Scop. and *Equisetum arvensis* L. (42.9 and 41.9%, respectively in Tartary and 55.0 and 25.9%, respectively in common buckwheat). The other notable weeds were *Setaria viridis*, *Poa annua*, *Malva verticillata*, *Chenopodium album* and *Amaranthus* sp. constituting 15.3 and 19.1%, respectively in Tartary and common buckwheat.

Tartary buckwheat

The data on weed counts, weed weights and weed control efficiency are shown in Table 1. Alachlor 1.50 kg/ha controlled weeds adequately and was as good as hand weeding in reducing the count of all the weeds. But hand weeding was superior to all the herbicides in reducing the total weed dry weight. Oxyfluorfen 0.20 kg/ha and pretilachlor 1.0 kg/ha were as effective as alachlor 1.50 kg/ha in reducing the count of *Digitaria* and other weeds. But overall performance of alachlor was better than oxyfluorfen and pretilachlor in reducing the dry weed weight. Sensitivity of *Digitaria* towards alachlor, oxyfluorfen and pretilachlor has been reported (Rao, 1983; Rana et al., 1999; Rana et al., 2003).

Imposition of hand weeding especially in pretilachlor and oxyfluorfen treated plots gave significant reduction in the count of *Digitaria*, other weeds and total weed dry weight and improved weed control efficiency consider-

Table 1. Effect of treatment on weed population, weed dry weight and weed control efficiency (WCE) in Tartary buckwheat

Treatment	Dose	Weed population (No. 0.50 m^{-2})			Weed dry weight (g m^{-2})			WCE (%)
	(kg/ha)	*Digitaria*	*Equisetum*	Other weeds	2001	2002	Mean	
Weedy		5.56	5.46	3.41	312.0	441.6	376.8	–
		(30.0)	(29.3)	(10.7)				
Hand weeding (HW)		2.07	3.36	1.00	24.0	28.0	26.0	93.1
		(4.0)	(10.3)	(0.0)				
Alachlor	1.50	2.34	3.64	2.37	69.2	70.8	70.0	81.4
		(5.0)	(12.3)	(4.7)				
Alachlor + HW	0.75	1.48	4.76	1.69	26.8	67.6	47.2	75.0
		(1.7)	(21.7)	(2.3)				
Pretilachlor	1.00	5.06	4.02	2.44	178.8	265.6	222.2	41.0
		(24.7)	(15.3)	(5.0)				
Pretilachlor + HW	0.50	1.54	3.92	1.27	41.2	117.6	79.4	78.9
		(2.0)	(14.7)	(0.7)				
Oxyfluorfen	0.20	3.36	4.09	2.99	208.0	262.4	235.2	35.6
		(10.0)	(16.0)	(8.0)				
Oxyfluorfeln + HW	0.10	1.13	5.50	1.00	56.0	117.6	86.8	77.0
		(0.3)	(29.7)	(0.0)				
LSD (P=0.05)		1.11	0.96	0.55	36.8	40.8	37.4	–

Data transformed to $\sqrt{x+1}$ transformation, the data in parentheses are the means of original values.

ably over their higher doses alone. Similarly, alachlor 0.75 kg/ha + hand weeding was superior to alachlor 1.50 kg/ha in reducing the count of other weeds and total weed dry weight in 2001. But generally there was build up of horsetail weed, *Equisetum* when hand weeding was super-imposed, as population of the weed increased following hand weeding in oxyfluorfen at 0.10 kg/ha and alachlor 0.75 kg/ha treated plots in comparison to their higher rates alone.

Owing to effective weed control, all treatments were superior to weedy check in influencing seed yield (Table 2). Alachlor 0.75 kg/ha + hand weeding and alachlor 1.50 kg/ha gave the highest two year mean seed yields, but were not significantly different from each other. Weed index expressing the reduction in yield due to the presence of weeds in comparison with hand weeding in the present study was minimum with alachlor 0.75 kg/ha + hand weeding (–12.0) followed by alachlor 1.50 kg/ha (–6.9). Similar to weed count and weed dry weight, hand weeding improved the efficacy of pretilachlor 0.50 kg/ha and oxyfluorfen 0.10 kg/ha in influencing seed yield and weed index over their higher rates alone. These were as good as hand weeding and alachlor 1.50 kg/ha in affecting seed yield.

Because of low cost of treatment and higher seed and straw yields, alachlor gave maximum marginal benefit cost ratio (MBCR) of 6.72. MBCR is defined as Net return due to weed control/cost of treatment = (Gross return due to weed control – cost of treatment)/cost of treatment. This was followed by oxyfluorfen 0.20 kg/ha (3.71) and alachlor 0.75 kg/ha + HW (3.65). However, all herbicidal treatments were superior to hand weeding in influencing MBCR.

In the present study, seed yield of Tartary buckwheat was negatively correlated with weed numbers of *Digitaria* (–0.868**, significant at 1% level), other weeds (–0.767*, significant at 5%) and total weed count (–0.829*) and with weed dry weight (–0.786*, 2001; –0.813*, 2002; –0.883**, mean). Seed yield was positively correlated with straw yield (0.815*, 2001; 0.847**, 2002; 0.883**, mean), plant height (0.761*, 2001; 0.777*, 2002; 0.819*, mean), branches/plant (0.818*, 2001; 0.506, 2002; 0.716*, mean) and seed yield/plant (0.853**, 2001; 0.642, 2002; 0.877**, mean) showing high degree of association as affected due to the presence of weeds. Seeds/plant (–0.874**), branches/plant (–0.773*) and straw yield (–0.714*) were negatively correlated with weed biomass. In the present study *Digitaria* seems to be more competitive as correlation between seeds/plant (–0.888**), and plant height (–0.856**) and the count of the weed were negative and significant. *Equisetum* sp. was the least competitive, as there was no significant correlation between yield, growth traits (plant height, branches, seed yield/plant, plant stand) and the weed counts. Seeds/plant and plant height were also significantly and negatively associated with other weeds and total weed count.

The total weed count and weed dry weight (X) were linearly correlated, at the significance level of 5 and 1%, respectively with the seed yield (Y) of Tartary buckwheat, the relationship was given by linear equation (Y=A + BX, A being intercept and B regression coefficient) as follow,

Weed count

$Y=2256–9.19X \quad (R^2=0.687)$

Weed dry weight

$Y=2175–1.51X \quad (R^2=0.854)$

The relationship between weed density and crop yield is largely sigmoidal (Zimdahl, 1980). The statistical analysis of the regression equations explain that with every g/m^2 increase in dry weight, the yield was expected to decrease by 0.88–2.13 kg/ha (mean 1.51 kg/ha). Decrease in yield due to the presence of one weed/0.5 m^2 was expected to be in the range of 2.99 to 15.39 kg/ha (mean,

Table 2. Effect of treatment on yield; weed index (WI), MBCR and threshold levels of weeds in Tartary buckwheat

Treatment	Dose (kg ha^{-1})	Seed yield (t ha^{-1})			WI (%)	Cost of weed control*	MBCR	Economic threshold
		2001	2002	Mean				
Weedy		1.21	1.94	1.58	20.3	–	–	–
Hand weeding		1.50	2.47	1.98	–	3382	1.89	20.4
Alachlor	1.50	1.93	2.31	2.12	–6.9	1441	6.72	8.7
Alachlor + Hw	0.75	1.72	2.72	2.22	–12.0	2898	3.65	17.5
Pretilachlor	1.00	1.51	2.19	1.85	6.6	1250	3.38	7.6
Pretilachlor + HW	0.50	1.71	2.36	2.03	–2.6	2960	2.22	17.9
Oxyfluorfen	0.20	1.45	2.21	1.83	7.6	1046	3.71	6.3
Oxyfluorfen + HW	0.10	1.76	2.36	2.06	–3.8	3008	2.42	18.2
LSD (P=0.05)		0.15	0.20	0.15		–	–	–

* Based upon prevalent prices in Rupees (1$=Rupees 46); Y=2256–9.19X (R^2=0.687)

9.19) with 95% confidence.

The economic threshold levels of weeds at the current prices of treatment application and the crop production on the basis of weed infestation (population) in Tartary buckwheat are given in Table 2. The economic threshold levels (number of weeds/unit area) with the weed management practices studied varies between 6.3–20.4 per 0.50 m^2. It is clearly indicated that any increase in the cost of treatment would lead to higher value of economic threshold, whereas an increase in the price of crop produce would result in lowering the economic threshold.

Common buckwheat

A critical perusal of the data (Table 3) revealed that counts of *Digitaria* and *Equisetum* were significantly lower following the application of alachlor 1.50 kg/ha. However, it was at par with hand weeding and alachlor 0.75 kg/ha + hand weeding in reducing the count of *Digitaria*. The other treatments also reduced the count of *Digitaria* significantly but they had no effect on *Equisetum*.

In general, the efficacy of all herbicides in reducing the dry weight of weeds was not very satisfactory, as indicated by weed control efficiency, which was 10.9, 50.4 and 55.7, respectively, for pretilachlor at 1.00 kg/ha, oxyfluorfen at 0.20 kg/ha and alachlor at 1.50 kg/ha. Hand weeding in integration with lower doses of the herbicides lowered the weed dry weight significantly and thus increased weed control efficiency over higher doses of the herbicides alone. Herbicides at low doses in integration with hand weeding were as effective as hand weeding in reducing the mean dry weed weight.

Generally all the herbicides were effective up to 30–35 days and alachlor especially was more effective. It has been established that alachlor at common rates controls weeds for 3–4 weeks only, but persists in soil for 6–10 weeks (Gupta, 1993). Persistence of pretilachlor in soil is for 20–50 days (Rana, 1997). The late occupants (later germinating weeds) though gained sufficient weight but did compete little as reflected in terms of yield (Table 4). Alachlor 1.50 kg/ha (WI, –41.7), alachlor 0.75 kg/ha + hand weeding (WI, –28.1) and oxyfluorfen 0.10 kg/ha + hand weeding (WI, –21.7) gave the highest two year mean seed yields, and the lowest weed index results, but were not significantly different from each other. In spite of controlling weeds effectively, the performance of hand weeding was poor in terms of yield. This may be due to the fact that weeds competed aggressively with buckwheat crop in the initial growth phases until the first weeding by 30–35 DAS was done. The WI, therefore, was negative for most of the treatments because of better performance with regard to seed yield over hand weeding. The increase in seed yield due to alachlor 1.50 kg/ha, alachlor 0.75 kg/ha + hand weeding and oxyfluorfen 0.10 kg/ha + hand weeding over weedy check was 97.6, 78.3 and 69.4%, respectively. Due to higher yields and low cost of treatment, alachlor 1.50 kg/ha resulted in

Table 3. Effect of treatment on weed population and dry weight in common buckwheat

Treatment	Dose (kg ha^{-1})	Weed population (No. 0.50 m^{-2})			Weed dry weight (g m^{-2})			WCE (%)
		Digitaria	*Equisetum*	Other weeds	2001	2002	Mean	
Weedy		5.53	3.85	3.04	350.4	245.0	297.7	–
		(29.7)	(14.0)	(10.3)				
Hand weeding (HW)		2.20	4.24	1.82	85.4	30.0	57.7	80.6
		(4.0)	(17.3)	(2.7)				
Alachlor	1.50	1.96	2.14	3.39	132.4	131.7	132.0	55.7
		(3.0)	(3.7)	(12.7)				
Alachlor + HW	0.75	2.34	4.15	2.14	80.2	29.0	54.6	81.7
		(4.7)	(16.3)	(5.3)				
Pretilachlor	1.00	4.69	3.46	3.70	252.4	278.3	265.3	10.9
		(21.0)	(11.0)	(13.3)				
Pretilachlor + HW	0.50	3.02	4.32	1.82	56.8	36.7	46.8	84.3
		(8.3)	(17.7)	(2.7)				
Oxyfluorfen	0.20	3.07	4.03	1.99	210.4	85.0	147.7	50.4
		(8.7)	(15.3)	(3.0)				
Oxyfluorfen + HW	0.10	3.04	4.47	2.03	55.4	33.3	44.4	85.1
		(8.3)	(19.0)	(3.3)				
LSD (P=0.05)		0.75	0.63	NS	15.9	38.5	35.4	

Data transformed to $\sqrt{x + 1}$ transformation, the data in parentheses are the means of original values.

Table 4. Effect of treatment on yield, weed index (WI), MBCR and threshold levels of weeds in common buckwheat

Treatment	Dose (kg ha^{-1})	Seed yield (t ha^{-1})			WI (%)	MBCR	Economic threshold
		2001	2002	Mean			
Weedy		0.473	0.514	0.494	28.2	–	–
Hand weeding		0.588	0.788	0.688	–	2.08	11
Alachlor	1.50	0.985	0.965	0.975	–41.7	9.37	4.7
Alachlor + HW	0.75	0.697	1.065	0.881	–28.1	3.78	9.4
Pretilachlor	1.00	0.548	0.727	0.638	7.3	1.86	4.1
Pretilachlor + HW	0.50	0.733	0.678	0.706	–2.6	2.40	9.6
Oxyfluorfen	0.20	0.672	0.788	0.730	–3.1	7.78	3.4
Oxyfluorfen + HW	0.10	0.745	0.928	0.837	–21.7	3.75	9.8
LSD (P=0.05)		0.075	0.187	0.162	–	–	–

$Y=1092-11.01X$ ($R^2=0.669$)

highest MBCR of 9.37. This was followed by oxyfluorfen 0.20 kg/ha (7.78), alachlor 0.50 kg/ha + hand weeding (3.78) and pretilachlor 0.50 kg/ha + hand weeding (2.40).

The seed yield was negatively associated with total weed count (–0.853**) and total weed dry weed (–0.709*) and positively associated with straw yield (0.849**), plant height (0.899**) and seeds/plant (0.75*). *Digitaria* was highly competitive with common buckwheat crop, its weed counts were significantly and negatively correlated with seed yield (–0.845**), straw yield (–0.895**), plant height (–0.905**), seed yield/plant (–0.847**) and final plant stand (–0.767*). Total weed count and dry weight were also negatively associated with straw yield, plant height and seed yield/plant.

The linear relationship between weed count (X) and seed yield (Y) of common buckwheat is given here as under,

$Y=1092-11.01X$ ($R^2=0.669$)

The equation explains that 66.9% variation in yield due to weed count could be explained by the regression equation. The further analysis indicated that decrease in yield per unit increase in weed count (1 weed 0.50 m^{-2}) is estimated to be between 11.01+7.7 kg/ha with 95% confidence. The economic threshold levels of weeds with the present rates of the treatments and the price of common buckwheat were estimated between 3.4–11 weeds 0.50 m^{-2}.

SUMMARY

Alachlor at 1.50 kg/ha was the best alternative to torturous and back-breaking hand weeding in Tartary as well as common buckwheat. The use of alachlor, oxyfluorfen or pretilachlor at lower rates, with subsequent hand weeding of the crop to get rid of left over weeds, may be more effective in providing acceptable weed control at economic rates. Presently, use of herbicides in buckwheat is nonexistent. But ever rising wages and fuel costs will give impetus to the farmers to switch over to herbicidal/integrated weed control. The farm children need to go to school instead of wasting their lives in weeding. The herbicidal or integrated weed management practices will let the farmer and farm women use their time thus spared in some other farming operations. Moreover, the fact, that alachlor and pretilachlor applied at herbicidal concentrations increase protein content, reducing and total soluble sugars (Sharma et al., 1978; Rana, 1997) has practical importance. Although the benefits of herbicides at sublethal (low) concentrations have been demonstrated (Rao, 1983), further work is required to effectively utilize this knowledge for the benefit of protein hungry world.

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Short Communication

Integrated effects of genotypes and fertility levels on leaf weevil infestation in buckwheat, *Fagopyrum* sp. under Western Himalayan conditions

Pankaj SOOD and Surendra S. RANA

Regional Research Centre, CSK Himachal Pradesh Krishi Vishvavidyalaya, Sangla 172 106, Kinnaur, HP, India

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Key words: Buckwheat, genotypes, fertility levels, *Strophosomoides kumaoensis*, B:C (benefit:cost) ratio

ABSTRACT

A field experiment was conducted at Sangla, Himachal Pradesh, India to study the effect of fertility schedules ($N_{15}P_{15}K_{15}$, $N_{40}P_{40}$, $N_{40}P_{40}K_{20}$ and $N_{60}P_{40}K_{20}$) and buckwheat genotypes on the incidence of leaf weevil, *Strophosomoides kumaoensis* Aslam. The trial included PRB-9001 a Tartary buckwheat line (*F. tataricum* Gaertn., phafra) and two common buckwheat (*F. esculentum* Moench, ogla) lines PRB-1 and OC-2. Common buckwheat genotypes were found to be more sensitive to weevil infestation when compared to Tartary buckwheat. Incorporation of potash (15–20 kg/ha) in the fertility schedule helped the buckwheat crop in avoiding weevil damage to a limited extent. The grain yield averaged, 1055, 527 and 435 kg/ha for PRB-9001, PRB-1 and OC-2, respectively. The results from this study suggest an application of 40 kg N, 40 kg P and 20 kg K/ha for reduced incidence of leaf weevil and for a more stable and sustainable productivity and higher profitability.

INTRODUCTION

Buckwheat is one of the most important and nutritious staple food crops of mountain regions. It is the only crop which can be grown at altitudes up to 4500 m (Joshi and Paroda, 1991). The seeds (achenes) are generally classified as a pseudo-cereal and the crop is cultivated both as a grain and as a leafy vegetable. Buckwheat has an advantage over cereals due to its high flavonoid content, particularly rutin, which is noted for its therapeutic effect for the treatment of blood vessel fragility with accompanied hypertension. It is also essential for a healthy immune system and decreased susceptibility to ontological diseases (Nikitchuk, 2000). The seed possesses a high lysine content which is an important essential amino acid in human health (Anonymous, 1979). Fertilizers play a major role for improving productivity. The response of buckwheat to fertilizers (Saini and Negi, 1998; Bogdanovic, 1987; Frolov, 1973; Munitsa, 1976) was found to vary with the genotype (Baburkova et al., 1999, 2000). However, excess fertilization, especially of nitrogen, promotes succulence and excessive vegetative growth and encourages a microenvironment that is favorable for pests. Therefore a balanced nutrition regime is of utmost importance in minimizing plant stress, susceptibility to pests and in maintaining good plant health. The leaf weevil, *S. kumaoensis* has been recorded as a major pest of buckwheat under Northern India conditions (Sood et al., 2003). The adult weevil damages the emerging seedlings, the young and tender leaves and the growing points, whereas the grubs feed on the internal root tissues. The present investigation was undertaken to study the integrated effects of nutrients and genotypes on the incidence of this dreaded pest.

MATERIALS AND METHODS

Three genotypes (PRB-9001, PRB-1 and OC-2) and four fertility levels ($N_{15}P_{15}K_{15}$, $N_{40}P_{40}$, $N_{40}P_{40}K_{20}$ and $N_{60}P_{40}K_{20}$) were arranged in a strip plot design with three replications and study was conducted at Sangla (2590 m above msl). The genotype PRB-9001 was Tartary buckwheat and PRB-1 and OC-2 were common buckwheat. The crop was sown on the third week of June at an inter-row spacing of 30 cm at a rate of 40 kg seed/ha. Alachlor at 1.50 kg/ha was applied as pre-emergence treatment for weed control one hour after sowing. The remainders of the management practices were followed in accordance with the recommended package of practices. The test site soil was sandy loam, neutral in reaction (pH, 6.9) with an organic carbon content of 0.69; N, 196 kg/ha; P, 16.4 kg/ha and K, 167 kg/ha. The climate of the region is characterized by moderate summers and cool winters.

Observations on weevil infestation were recorded immediately after germination and at weekly intervals thereafter. A one square meter area in each plot was randomly selected and marked. The number of infested plants and the total plants in the marked area were recorded to determine the percent weevil infestation.

The yield and yield contributing characters were

recorded at harvest time from a net plot area of 10.5 m^2. The economics (benefit:cost ratio) of the treatments was computed based upon prevalent market prices.

RESULTS AND DISCUSSION

Genotypes

The seed yield of the Tartary buckwheat genotype, PRB-9001 was significantly higher (at the level of 0.05%) when compared to that of PRB-1 and OC-2, the common buckwheat genotypes (Table 1). This was mainly ascribed to significantly higher yield attributes. One important factor causing low productivity in common buckwheat is self incompatibility, a characteristic of the species originating from its dimorphic heterostylic system (Adachi, 1986) *viz.* short styled (thrum) and long styled (pin) flowers. The yield of common buckwheat has been shown to be strongly affected by the flower fertilization rate which is usually low (Inoue et al., 2002) in this cross pollinated species as compared to Tartary buckwheat which is a self-pollinated species. Furthermore, weevil infestations were significantly higher in the common buckwheat genotypes as compared to the Tartary buckwheat genotype. The higher incidence of weevil in the common buckwheat genotypes may possibly be attributed to its sweeter taste, as compared to Tartary buckwheat, which is slightly bitter in taste possibly due to its high rutin content (Kitabayashi et al., 1995). The differences between the common buckwheat genotypes, with respect to weevil infestation, were statistically non-significant, although PRB-1 recorded a slightly higher weevil infestation. The line PRB-1 produced a higher yield with a higher fertilization rate as evidenced from a higher number of seeds/plant which resulted in higher per plant yield. However, the straw yield did not differ significantly between the two genotypes. Variation in buckwheat yield, due to genotypes, has been well documented (Baburkova et al., 1999, 2000). The genotype PRB-9001 was found to produce significantly higher net returns and B:C (benefit:cost) ratio because of its higher productivity. It was followed by PRB-1.

Fertility levels

The addition of potash at 20 kg/ha over the recommended dose (40 kg N + 40 kg P_2O_5) significantly reduced the weevil infestation. The role of potassium in buckwheat nutrition has been well documented (Frolov, 1973; Ivanov and Bondarchuk, 1976). The seed and straw yields were also higher following the addition of 20 kg K_2O/ha to the recommended dose. Earlier, Ram and Gupta (1992) had reported a lower incidence of leaf caterpillar in soybean and mustard aphid, *Lipaphis erysimi* (Kalt) on Indian mustard, respectively, following potash application. An increase in nitrogen from 20 kg to 30 kg/ha (basal dose) increased the weevil infestation and decreased the yield significantly. Apart from the higher weevil infestation, the decrease in yield at the higher level may be due to the operation of the Law of Diminishing Returns (Tisdale et al., 1990). Moreover, in this treatment ($N_{60}P_{40}K_{20}$), it was found that when a half of the N was applied at sowing time it was inhibitory to the germination, emphasizing that any additional N should be applied in split applications. Additional phosphorus fertilization was found to have no significant influence on weevil infestation. Rawat and Singh (1983) had also observed that phosphorus fertilization did not influence the severity of aphid infestations.

Interaction

The integrated effects of genotypes and fertility levels

Table 1. Effect of genotypes and fertility levels on yield contributing characters, yield and economics of buckwheat

Treatment	Leaf weevil infestation* (%)	Seed yield (kg/ha)	Straw yield (kg/ha)	Yield Plant ^{1}	Seeds Plant^{-1}	1000-seed weight (g)	Net returns (Rupees)	B:C ratio
Genotypes								
PRB-9001	56.71 (48.99)	1055.0	1602.8	2.85	136.5	20.81	15065	1.90
PRB-1	75.18 (60.43)	527.0	847.3	2.23	109.2	20.52	9331	1.12
OC-2	70.91 (57.86)	435.0	986.0	1.74	84.5	20.65	7408	0.90
LSD (P=0.05)	9.81 (5.78)	80.9	161.1	0.22	10.6	NS	1596	0.21
Fertility levels								
$N_{15}P_{15}K_{15}$	64.91 (53.98)	572.0	981.3	1.74	84.4	20.75	8316	1.12
$N_{40}P_{40}$	80.28 (63.95)	693.0	1222.3	2.46	129.0	19.00	11381	1.39
$N_{40}P_{40}K_{20}$	57.66 (49.56)	882.0	1433.3	2.79	128.9	21.89	16567	1.99
$N_{60}P_{40}K_{20}$	67.54 (55.57)	543.0	944.3	2.10	100.0	21.00	6140	0.73
LSD (P=0.05)	11.05 (7.38)	74.4	107.4	0.21	10.2	0.57	2129	0.24

* Figures in parentheses are arcsine transformed values.

Table 2. Integrated effects of genotypes and fertility levels on yield and economics of buckwheat

Fertility levels	Genotypes		
	PRB-9001	PRB-1	OC-2
	Seed yield		
$N_{15}P_{15}K_{15}$	941	386	389
$N_{40}P_{40}$	1056	556	467
$N_{40}P_{40}K_{20}$	1222	833	589
$N_{60}P_{40}K_{20}$	1000	333	296
LSD (P=0.05)		95.6	
	Weevil infestation* (%)		
$N_{15}P_{15}K_{15}$	51.07 (45.60)	76.93 (61.30)	66.73 (55.04)
$N_{40}P_{40}$	74.27 (59.57)	83.80 (66.54)	82.77 (65.72)
$N_{40}P_{40}K_{20}$	48.27 (43.98)	65.93 (54.29)	58.77 (50.41)
$N_{60}P_{40}K_{20}$	53.23 (46.84)	74.03 (59.58)	75.37 (60.28)
LSD (P=0.05)		NS	
	B:C ratio		
$N_{15}P_{15}K_{15}$	1.83	0.77	0.76
$N_{40}P_{40}$	1.90	1.23	1.05
$N_{40}P_{40}K_{20}$	2.28	2.23	1.45
$N_{60}P_{40}K_{20}$	1.59	0.26	0.33
LSD (P=0.05)		0.38	

* Figures in parentheses are arcsine transformed values.

with respect to weevil infestation were found to be non-significant; although significant variation in seed yield and B:C ratio was recorded. The yield of each genotype was significantly higher when fertilized with 40 kg N+40 kg P_2O_5+20 kg K_2O/ha. The yield decreased when 20 kg more N/ha was given i.e. $N_{60}P_{40}K_{20}$. At each fertility level the yield of PRB-9001 was significantly higher than either of the common buckwheat genotypes. The line PRB-1 appeared to be more responsive to K than either of the other genotypes evaluated, as evidenced by the per cent yield increase in this genotype with the application of $N_{40}P_{40}K_{20}$ over $N_{40}P_{40}$ which was remarkably higher (49.82%) as compared to 15.72% in PRB-9001 and 26.12% in OC-2 over the same fertility schedule. The genotype PRB-9001, when fertilized at the $N_{40}P_{40}K_{20}$ fertility level resulted in a maximum seed yield of 1222 kg/ha. However, due to the higher price of common buckwheat seed, when PRB-1 was fertilized at the same fertility schedule ($N_{40}P_{40}K_{20}$) it resulted in a B:C ratio which was statistically at par with PRB-9001 when fertilized at the $N_{40}P_{40}K_{20}$ fertility level. The results from this study demonstrated that 40 kg N, 40 kg P_2O_5+20 kg K_2O was the optimum fertility schedule for stable and sustainable yields and resulted in a lower weevil infestation, irrespective of the cultivars/genotypes evaluated.

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Short Communication

Preliminary studies on mites (*Acarina*) infesting stored grain and other buckwheat products

Wit CHMIELEWSKI

Department of Bee Products, Apiculture Division, Research Institute of Pomology and Floriculture, Kazimierska 2, 24-100 Pulawy, Poland

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ABSTRACT

Acarological analyses of samples of various stored buckwheat products (cereals/groats, grain/seeds, hay/straw, post harvest sweepings, black pudding, honey) collected from farms (barns, granaries and other husbandry buildings), shops and storehouses, were conducted. The results obtained show that some of them were infested and contaminated with over 20 mite species. The species found belonged mainly to the synanthropic acarid and glycyphagid mites (families: Acaridae, Glycyphagidae), often occurring in storehouses, infesting stored food-stuffs and other materials. The most frequent and numerous of them were the following species: *Acarus farris* (Oud.), *Acarus immobilis* Griffiths, *Acarus siro* L. *Tyrophagus longior* (Gerv.), *Tyrophagus putrescentiae* (Schr.), *Glycyphagus domesticus* (De Geer), *Lepidoglyphus destructor* (Schr.). They are commonly considered to be known pests of economic and sanitary importance. Mites of other groups (*Bdellidae*, *Macrochelidae*, *Tydeidae*, *Anoetoidea*, *Tarsonemoidea*, *Oribatida*, *Uropodida*, *Gamasida*, *Parasitida*) were also observed. Acaroid and other phytophagous mites infesting buckwheat products were usually accompanied with predatory species, such as *Cheyletus eruditus* (Schr.), *Acaropsis sollers* Rohdendorf, *Melichares tarsalis* (Berl.) and *Bdella* sp., which are natural enemies of other stored products mites, insects and other small arthropods, which usually occupy similar habitats and colonize the same products.

INTRODUCTION

An increase of interest in the cultivation of buckwheat and the production of buckwheat grain and other derivative products has increased the necessity of protecting of these stored goods against various pests of stored food and storage areas. Foreign literature and Polish scientific and popular publications concerning mites as stored product pests, and their economic and sanitary importance as factors causing allergies and other diseases, are very numerous (among others: Boczek, 1980; Boczek and Czajkowska, 2003; Chmielewski, 1971; Fain et al., 1988; Hughes, 1976; Rosicky et al., 1979). Unfortunately there are no faunistic data about the pests of buckwheat products, except for one paper on the occurrence of mites in Polish storehouses, which is a notice regarding some collected samples of buckwheat groats infested with house mites, *Glycyphagus domesticus* (De Geer) and *Lepidoglyphus destructor* (Schr.) (Boczek and Golebiowska, 1959). Results of the author's earlier studies on the biological observations and mode of life and experiments on the life history, longevity, fecundity and other biological parameters of acaroid mites (*Acarus siro* L., *Tyrophagus putrescentiae* (Schr.), *Caloglyphgus berlesei* (Michael), *Rhizoglyphus echinopus* (F. et R.), *Carpoglyphus lactis* (L.), *G. domesticus*, *L. destructor*) show that buckwheat products are an attractive and effective food for these pests (Chmielewski, 1998, 1999, 2000, 2001a, b, 2002a, b, 2003). Recent studies conducted on the fauna associated with buckwheat products also confirm the hypothesis that various groups of arthropods, mainly acaroids and insects, are potential and real pests (Chmielewski, 2004).

The aim of the present study was to document species composition and the intensity of occurrence of acarofauna in stored buckwheat products to broaden our knowledge about the subject.

MATERIAL AND METHODS

Material was collected during periodic visits to farms, shops and storehouses. Samples (100–500 g weight) representing various kinds of buckwheat products suspected of infestation with pests, i.e. grain for consumption, sowing material (seed), post harvest sweepings (dust, litter, hulls), hay/straw (dry leaves, stalks and other plant material), kernels (groats), black pudding (a type of sausage) and buckwheat honey, were analysed using the methods which have been described earlier (Chmielewski, 1969, 1971, 1995). Buckwheat grain and other friable (loose) and fine granulated products were sifted with the use of laboratory strainers. The same sifter sets were used in

laboratory tests of similar materials, e.g. dried medicinal herbs, sugar beet or grass seeds etc. The fine granulated fraction which was obtained this way from each sample was observed under a low magnification microscope. Products of any other consistency, i.e. solid (pudding) and liquid (honey), were analyzed immediately (without sifting) under the microscope or with the help of a filtration method (Chmielewski, 1995a, b).

The intensity of any infestation and the contamination of products with pests (number of living+dead pest specimens per weight unit of product) was calculated using a 3-degree scale (numerous criteria) used in earlier similar studies on other stored products, e.g. medicinal herbs (Chmielewski, 1972a, b), i.e. as follows: Ist—1–2, IInd —3–5, IIIrd degree>5 mite specimens per 5 g of product sample.

Any mites and small arthropods which were found were picked from the material examined and identified under a phase-contrast microscope using the keys and descriptions in available literature (Boczek, 1980; Fain et al., 1988; Hughes, 1976; Kielczewski et al., 1967; Rosicky et al., 1979; Turk and Turk, 1957; Zakhvatkin, 1941).

RESULTS AND DISCUSSION

Faunistic observations of arthropods in buckwheat products showed that stored product mites were the dominant group of pests infesting them. The percentage of samples infested with mites was ca. 84.3% and were comparatively high (by comparison to those found in other kinds of stored products) (Chmielewski, 1971a, b, 1975). The intensity of infestation of buckwheat samples with these pests (living and dead specimens), as calculated using the three degree scale, was as follows: I—6.3%, II —15.0%; III—63.0% of the total number of infested samples, while 15.7% of the total analyzed samples were not infested (free of mites). A large number of the analyzed samples was infested to a greater (IIIrd) degree, i.e. more than 5 mite specimens per 5 g of product (Table 1).

The mites found in the buckwheat products included over 20 species (Table 2). The majority of them were synanthropic, a free living species, which are usually known as invaders and inhabitants of flats, storehouses, barns and other husbandry buildings. They usually form miscellaneous populations consisting of species representing various systematic groups, which are especially numerous in damp post harvest sweepings, straw, hay, hulls, seeds, cereals and other products stored under primitive conditions (high humidity, temperature, unsuitable rooms and containers).

The most frequent and numerous mites found during the present examination belonged to two families of the acaroid group (*Acaroidea*), i.e. acarids (fam. *Acaridae*): *Acarus farris* (Oud.), *Acarus immobilis* Griffiths, *A. siro*, *Tyrophagus longior* (Gerv.), *T. putrescentiae*) and glycyphagids (fam. *Glycyphagidae*): *G. domesticus*, *L. destructor*, which are commonly known as pests of economic and sanitary importance in stored products. These species were usually found as specimens in all the known developmental stages, i.e. eggs, larvae, nymphs and imagines. Hypopodes (heteromorphic deutonymphs) of some species (*L. destructor*, *G. domesticus*, *Acarus* sp., *Rhizoglyphus* sp., *Caloglyphus* sp., *Calvolia* sp., *Histiostoma* sp., *Myianoetus* sp., *Anoetidae*), were also observed. Of these hypopus specimens of glycyphagids (*Glycyphagidae*) were found the most often. The hypopal forms serve in the survival and spread of these species, especially during unfavourable life conditions (e.g. humidity, temperature).

Specimens representing mites of other groups (*Bdellidae*, *Macrochelidae*, *Tydeidae*, *Anoetoidea*, *Tarsonemoidea*, *Oribatida*, *Uropodida*, *Gamasida*, *Parasitida*) were also observed; some of them occurring in significantly lower numbers than the acaroids or were found as sporadic contamination only. Some of these, e g. soil or plant-feeding mites (oribatids, gamasids, uropo-

Table 1. Intensity of infestation and contamination of stored buckwheat products (%) with mites (*Acarina*); infestation scale (in degrees): Ist—1–2, IInd—3–5, IIIrd degree—>5 mite specimens per 5 g of product sample

Products	Number of samples		Infestation of samples (%)			
			Total	Degrees		
	Collected	Infested	I+II+III	I	II	III
Grain/seed	22	20	90.9	4.6	13.6	72.7
Hay/straw	13	13	100.0	7.7	15.4	76.9
Post-harvest sweepings	64	63	98.4	6.2	17.2	75.0
Kernels/groats	6	2	33.3	0.0	0.0	33.3
Black pudding	10	5	50.0	10.0	20.0	20.0
Honey	12	4	33.3	8.3	8.3	16.7
Total	127	107	84.3	6.3	15.0	63.0

Table 2. List of mite species (*Acarina*) found in infested buckwheat products samples and the degree of infestation (%); total number of examined samples: collected—127, infested—107 (84.3%); infestation scale (in degrees): I—1–2, II—3–5, III—>5 mite specimens per 5 g of product sample

Mites	Number of infested samples	Infestation (%)			
		Total	Degrees		
		84.3	I	II	III
Acarus farris (Oud.)	8	7.5	25.0	50.0	25.0
Acarus immobilis Griffiths	5	4.7	60.0	40.0	0.0
Acarus siro L.	13	12.1	30.8	30.8	38.4
Tyrophagus longior (Gerv.)	21	19.6	28.6	28.6	42.8
Tyrophagus putrescentiae (Schr.)	12	11.2	25.0	50.0	25.0
Glycyphagus domesticus (De Geer)	35	32.7	14.3	22.9	62.8
Lepidoglyphus destructor (Schr.)	31	29.0	16.1	22.6	61.3
Ctenoglyphus plumiger (Koch)	2	1.9	100.–	0.0	0.0
Gohieria fusca (Oud.)	1	0.9	100.–	0.0	0.0
Carpoglyphus lactis (L.)	4	3.7	25.0	50.0	25.0
Dermatophagoides pteronyssinus (Trt)	1	0.9	100.–	0.0	0.0
Tarsonemus fusarii Cooreman	16	20.0	31.2	31.3	37.5
Ameroseius plumigerus (*Oud.*)	7	6.5	14.3	57.1	28.6
Ameroseius plumosus (*Oud.*)	5	4.7	60.0	0.0	0.0
Melichares tarsalis (Berl.)	7	6.5	57.1	28.6	14.3
Acaropsis sollers Rohdendorf	11	10.3	72.7	27.3	0.0
Cheyletus eruditus (Schr.)	22	20.6	27.3	40.9	31.8
Cheyletia flabellifera (Michael)	1	0.9	100.–	0.0	0.0
Uroobovella marginata (C.L. Koch)	3	2.8	66.7	0.0	33.3
Unidentified species:					
Acaroidea	16	12.6	37.5	43.8	18.7
Anoetoidea	2	1.9	100.–	0.0	0.0
Aceosejidae	3	2.8	66.7	33.3	0.0
Bdellidae	24	22.4	37.5	33.3	29.2
Cheyletidae	6	5.6	83.3	16.7	0.0
Laelaptidae	5	4.7	60.0	20.0	20.0
Macrochelidae	2	1.9	100.–	0.0	0.0
Parasitidae	3	2.8	100.–	0.0	0.0
Tarsonemidae	10	9.0	25.0	25.0	50.0
Trombidiidae	2	1.9	100.–	0.0	0.0
Tydeidae	19	17.6	36.8	26.3	36.9
Gamasida	3	2.8	66.7	33.3	0.0
Oribatida	31	30.0	58.1	19.3	22.6
Uropodida	4	3.7	50.0	50.0	0.0
Acarina (undetermined specimens)	14	13.1	78.6	14.3	7.1

dids) appeared to be quite incidental contaminants of the stored buckwheat products.

The products most often colonized with mites (especially *G. domesticus*, *L. destructor*) were post harvest sweepings, hay, hulls and straw. Seed, groats and other grain products were also infested with glycyphagids (fam. Glycyphagidae), but mainly with acarids (fam. Acaridae, e.g. *Acarus* spp., *Tyrophagus* spp.). Whereas *C. lactis* (fam. Carpoglyphidae) was a typical pest of honey, other mite species, mainly *G. domesticus*, were found as

contaminants (dead specimens only) in this product. Living mite specimens in all stages were observed on the surface of old and damp buckwheat black pudding. Damp sweepings, remnants and products in barns or other husbandry buildings, stored usually under primitive conditions, were usually infested to a greater degree (II–III). Buckwheat seed, kernels, other grain products and foodstuffs stored under controlled low temperature and humidity, in hermetic containers and in modern storehouses, were sporadically contaminated and at the lowest degree (single dead mite specimens) or were usually free of all pests.

The species mentioned above were often accompanied with predators, such as *Cheyletus eruditus* (Schr.), *Acaropsis sollers* Rohdendorf, *Melichares tarsalis* (Berl.), Bdella sp., which are natural enemies of other stored products mites and small arthropods and usually occupy similar habitats and settle on the same products.

Insects and other arthropods, isopodids and arachnids, e.g. single spiders or false scorpions, found in buckwheat products, have been listed in a separate paper (Chmielewski, 2004).

CONCLUSIONS

The species composition of the acarofauna and the relationship between buckwheat products and the mites colonizing them are still insufficiently or completely unknown. The results obtained in the present study are evidence that the species composition of acarofauna in buckwheat is numerous and very diverse.

They show that the continuation of faunistic and bioecological research on mites associated with stored buckwheat products appears to be well-founded, however, the economic and sanitary importance of these pests, e.g. their role as vectors in the spread of saprophagous and pathogenic microorganisms (bacteria, fungi), require further investigation. Results of these studies would have cognitive and practical aspects and would provide a good basis for further investigations on prophylaxis/control during storage for the protection of buckwheat grain and other goods against stored products pests.

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Review Paper

Buckwheat production, utilization, and research in China

Yingjie WANG* and Clayton G. CAMPBELL

Kade Research Ltd. 135 13 Street, Morden, Manitoba, Canada R6M 1E9

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ABSTRACT

A Chinese semi-annual journal called "Buckwheat Trend" was initiated in 1984. This journal normally publishes three types of papers, i.e. reviews articles, research papers within China and a few translated research papers from around the world. It also issues announcements and news related to buckwheat events. This journal ties buckwheat workers together within China and gives them updated information on buckwheat all over the world. However, English publications from this journal are very limited. This review briefly summarizes the publications from "Buckwheat Trend" during 1994–2003 in an attempt to give an overall view of buckwheat production, utilization and research in China.

GENERAL INTRODUCTION

China is the most populated country with one fifth of the world's population. Buckwheat originated in China which has the longest cultivation history of it in the world. Presently China is the world's second largest buckwheat producer and the largest buckwheat exporter. Today, China's economics is rapidly increasing and it also has become a member of the WTO, which will promote buckwheat research, production and marketing.

Buckwheat organizations in China

The Buckwheat Association of China was founded in 1989 (Lin, 2002). It's members come from all over the country and meet together regularly to exchange information on buckwheat research, results of national yield trails, production and utilization trends, announce buckwheat events, discuss regulations or renew the editing board of the Journal "Buckwheat Trend". The Association published the first volume on Chinese buckwheat research in 1989. Buckwheat research in China is conducted at many different research units such as the Chinese Academy of Agricultural Sciences at the national level; provincial agricultural academies such as Shanxi and the Inner Mongolia Academy of Agricultural Sciences; universities at both the national and provincial levels, such as the Northwest Agricultural University, Shanxi Agricultural University, and Shanxi University; and numerous agricultural and research institutes at the city, prefecture and county levels.

Buckwheat production and export in China

Buckwheat is a minor crop in China. It grows over large regions with most production in the cooler or mountain areas (Liu et al., 2003). Common buckwheat grows in more than 16 provinces/autonomous areas with a predominance in the northern part of the country (76%) at an altitude of 600–1,500 m., including Inner Mongolia and in the northwest in the provinces of Shannxi, Shanxi, Ganshu, and Ningxia. Tartary buckwheat grows in more than 11 provinces/autonomous areas with predominance on the northwest Loess Plateau and the southwest plateau of Yunan and Sichuan at an altitude of 1,200–3,000 m (Cao et al., 1998; Lin, 2000; Li et al., 2001a; Liu et al., 2003). *Fagopyrum tataricum* with non-winged seeds is widely grown, while *F. tataricum. var. alata* with winged seeds (Zhao et al., 1999) is produced in very limited areas such as Liangshan Autonomous Prefecture of the Yi nationality in Sichuan province and in northern China (Gu, 1999). Production of buckwheat in China has been approximately 1.33 million hectares per year (Li et al., 2001b; Liu et al., 2003) which includes 900,000 ha of common buckwheat and 400,000 ha of Tartary buckwheat. The total yield is approximately 500,000 tons yearly, therefore China is the second largest buckwheat producing country in the world (Cao et al., 1999; Liu, N.Q., 2002; Chai et al., 2001; Wang, G.Y., 2002; Shi, 2002).

Approximately 60–70% of the buckwheat produced is consumed domestically, 20% is utilized as feed, and 10% is exported (Xu et al., 2000; Feng et al., 2003). China ranks number one for buckwheat export in the world with approximately 100,000 tons yearly, of which approximately 80,000–90,000 tons is exported to Japan (Wang, G.Y., 2002; Chai et al., 2001; Zhang, 2002). The international trade price of buckwheat is two to three times

*Corresponding author. Tel: 1-204-822-7235, Fax: 1-204-822-5960, E-mail: kaderes@mts.net.

higher than that of other Chinese grain crops. However, compared with buckwheat from other countries, Chinese buckwheat has a lower trade price due to its low quality. For example, Chinese buckwheat is one half the price of American and Canadian buckwheat and one third the price of Australia buckwheat (Shi, 2002; Zhang, 2002). The low quality is caused by non-professional production, poor cultural techniques as well as by different production styles by which buckwheat is produced by thousands of small farmers resulting in variety mixtures and an impure product (Zhang, 2002).

As improved processing techniques developed during the 1990's, China started to export buckwheat products or semi-finished buckwheat products, such as groats, toasted groats, buckwheat tea, flour, noodle, and vinegar, which are estimated at approximately 20,000 tons yearly (Lin, 2000; Zhang, 2002). In the domestic market, buckwheat products such as noodles, biscuits, vinegar, and wine are the customer's favorite choice due to their health benefits. The price obtained for the buckwheat products is 3–5 times higher than similar products made from other crops (Liu et al., 2003).

Ecological regions of buckwheat in China

China has been divided into four ecological regions based on buckwheat distribution, variety characteristics, and local planting systems: (1) North spring buckwheat which includes approximately 50–60% of the common buckwheat in the total country; (2) North summer buckwheat which includes 15%–20% of the common buckwheat of the total country; (3) South fall and winter buckwheat with sporadic plantings; and (4) Southwest high plateau spring and fall buckwheat which includes most of the Tartary production in China (Feng et al., 2001). Buckwheat grows well at high altitudes with high sunlight intensity, and large differences between day and night temperature in such provinces as Shanxi, Gansui (Xu et al., 2000; Zhang and Yang, 2001). Drought and frost are the two largest natural disasters for buckwheat production in China (Zhang and Yang, 2001).

Buckwheat culture in China

Buckwheat has been named as "the king of cereals" in China due to its high content and high quality of proteins, lipids and starch, and unique flavonoids as compared with the cereals (Li et al., 2001). The medicinal value of buckwheat has been recorded in many historical Chinese medical books including «Ben Cao Gang Mu», «Qian Jin Yao Fang», and «Ben Ciao Shi Jian» (Li et al., 2001; Fan, 2002; Wang et al., 2002a).

Tartary buckwheat plays a very important role in the life of Yi nationality that live in Liangshan Autonomous Prefecture (below the provincial level) in Sichuan province (Li et al., 1999b; Zhao et al., 1999; Zhao, 2000). They worship their buckwheat fields during their holiday celebration as they consider buckwheat as their god and mother. Buckwheat is their main staple food and is used as gifts in various important events such as traditional holidays, weddings, and funerals.

GERMPLASM COLLECTION AND EVALUATION

Collection and evaluation of buckwheat landraces and varieties

In total, 2,785 buckwheat accessions have been collected in China. These have been planted, evaluated, and catalogued in a list of national germplasm and are stored in long term storage (Lin, 2000). More than 85% of the buckwheat in China is distributed in a long narrow strip running from the northeast to the southwest (Inter Mongolia–Hebei–Shanxi–Shannxi–Gansui–Sichuan–Yunan) (Cao et al., 1998). These collections consist of 67% common buckwheat and 33% Tartary buckwheat. A total of 879 Tartary collections were from 16 provinces/autonomous areas of which Sichuan, Yunan, and Shanxi contain 46%, 19.06%, and 12.86%, respectively (Lin, 2000).

Wang and Ma (1997) analyzed the isozyme patterns of 20 common and Tartary buckwheat samples which represented 12 provinces/autonomous areas. The SOD isozyme had three strong active bands, but Tartary buckwheat only shared one of them. The EST isozyme varied greatly with three types of mobility, fast, slow, and absent types, while MDH and GDH varied slightly between the two species and within the species. Both isozyme analysis and RADP marker analysis showed that common buckwheat has greater variation than that of Tartary due to cross-pollinating in common buckwheat (Wang et al., 1995; Zhou et al., 1996).

Collection and evaluation of wild species

Yunan province, which is located in the Southwest of China, is the origin and center of buckwheat. Wang Lihua and her colleagues from the Yunan Academy of Agricultural Sciences have collected and evaluated buckwheat species in Yunan (Wang et al., 2000, 2002) supported by a Natural Scientific Fund from Yunan Province. They made six trips during 1998 to 2001 and covered more than 50 counties. Wild buckwheat grows at an elevation of 500–4,000 m with most of it being found at approximately 2,000 m in elevation. Twelve species or subspecies have been reported to be found in this province including *F. cymosum, F. urophllum, F. statice, F. gracilipes, F. gracilipes var. odontopterum, F. leptopodum, F. leptopodum var. grossii, F. lineare, F. esculentum ssp. ancestrale, F. tataricum ssp potanini, F. homotropicum, and F. x* (Wang et al., 2002). Among them, *F. cymosum*

and *F. gracilipes* was distributed in all 17 regions of the province. The former grows in shady and wet places such as on the bank of rivers, open lands, low banks between fields, shadily sloping places, roadsides, and under bushes or shrubs. The later can be found in uncultivated lands with dry and poor soil, on mountain slopes, and on farm lands, especially in corn and buckwheat fields as a weed. *Fagopyrum urophllum* is the third widely distributed species in Yunan and is following by *F. leptopodum*. Western and central Yunan are the two distribution centers of wild buckwheat species in this province. An efficient method for DNA extraction and reactions for studying these wild species has been established (Wang and Ye, 2002).

Collection and evaluation of germplasm from Tibet

The collection and evaluation of Tibet buckwheat germplasm was organized by the Institute of Crop Germplasm in the Chinese Agricultural Academy of Sciences (Lu, 1995; Wang et al., 1996). The team collected more than 200 samples during 1981–1984. Sixty-six samples were selected for evaluation, including morphology, isozymes of esterase and other enzymes, cytology, and seed quality in the following five years and were supported by a national project. Buckwheat is widely cultivated in Tibet except in the pure pasture areas (northeast and northwest). Based on morphological characters, cultivated buckwheat in Tibet can be divided into seven types, including two types of common buckwheat (red and white flowers) and five types of Tartary buckwheat (gray hull, black hull, winged seed, black rice, and gray rice). The wild buckwheat found here included annual Tartary buckwheat and perennial buckwheat (*F. cymosum*). It was found that in total, 53 zymograms were clustered into six groups. Cluster analysis indicated that cultivated buckwheat was concentrated but the wild species were dispersed. Cultivated rice Tartary behaved similarly to the wild species, suggesting it is a primitive cultivated type. The chromosome length of common buckwheat (2n=2x=16) is 4.18–6.02 nm and the chromosomes have two satellites located on chromosome 2 and 5. Tartary buckwheat (2n=2x=16) has one satellite on chromosome 2. Rice Tartary was considered as a subspecies of Tartary buckwheat based on seed morphology and karyotype (Wang et al., 1996). Unlike Tartary buckwheat, rice Tartary doesn't have grooves on the seed hull and the hull has splits on each of the three sides which make it easy to dehull. The chromosome differences between Tartary and rice Tartary included chromosome length and the number of submetacentric chromosomes. Tartary buckwheat has three submetacentric chromosomes (2, 5, and 6) with an average length 2.28–4.76 nm, while rice Tartary has four submetacentric chromosomes (2, 3, 4, 6) with an average length 2.00–3.24 nm. *Fagopyrum cymosum* is a perennial species containing both diploids and tetraploids. The tetraploid form is an allotetraploid (2n=4x=32) with two small satellites on chromosomes 4 and 8 and an average length 2.54–4.34 nm. Quality analysis showed that Tartary buckwheat has higher protein content than common buckwheat. The protein was shown to have less variation than did the mineral content, such as Fe, Mn, and Ca, within a species.

BUCKWHEAT BREEDING

Buckwheat breeding in China did not begin until the early 1980's when the government started to collect and evaluate buckwheat germplasm (Chai et al., 2001). Up to 2000, eight common buckwheat varieties and two Tartary buckwheat varieties had been registered in different provinces. Three Tartary buckwheat varieties have passed national registration. Among them, one variety is tetraploid which was developed through colchicine treatment, three varieties were bred using radiation and the rest were developed through mass selection from local races and introduced germplasm. The national Buckwheat Yield Trials in China started in 1984 with three years in each cycle. The trial was conducted in 15 provinces/autonomous areas including 20 locations for common buckwheat and 18 locations for Tartary buckwheat. After preliminary yield trails at breeding locations, any new variety release is based on the National Yield Trials.

Breeding objectives and strategies

The objectives of common buckwheat breeding are the improvement of flour and nutrient quality, increase in seed set and lodging resistance. The objectives of Tartary buckwheat breeding are to increase flavonoid content, improve flour quality, improve resistance to lodging, and increase kernel weight (Chai et al., 2001). Li et al. (1999) proposed the ideal characters for Tartary buckwheat, i.e. plant height<100 cm, grain yield: plant yield above ground=1:1–1.2, thousand kernel weight 20.0–24.0 g, short internodes and compact plant habit, short growth period, and cold tolerance.

Two main problems, non uniform maturity and low seed set, impede the increase of buckwheat yield in buckwheat crop development (Tang and Zhao, 2002). Breeding for a determinate type to improve the uniformity of maturity was not efficient in Tartary as the realization of a large amount organic matter accumulation over a short period is very difficult. It was possible to improve yield through increased seed set, thousand kernel weight, the number of seeds developed on each plant, and resistance to seed shattering.

Breeding methods

Introduction: Several introduced varieties from Japan and

one from North America have been grown in China after further selection. Mei Guo Tian Qiao was introduced into China in 1988 by Guoyuan Agricultural Institute in Gansu province (Ma et al., 2002). The meaning of the name is America common buckwheat. This probably originated from Canada from the variety Mancan (author). After this introduced germplasm was selected for several years and tested in yield trails, it was released as a variety in 1995 in the Ningxa Autonomous Region with a 43% yield increase as compared to the local varieties. The average yield from 29 locations was 821 kg/ha (55 kg/mu) and the highest yield was 1,890 kg/ha (126 kg/mu). Another common buckwheat variety, Ping Qiao No. 2, was selected from a Japanese collection (Chang et al., 2003).

Mass selection or single plant selection: Mass selection is the primary method for buckwheat breeding in China and produces most varieties. The base populations can be a local race, a variety, or a mixture of collections. For example, a new variety, Feng Huang Tartary, was developed from an old variety, Jiu Jiang Tartary, which had degenerated and was impure (Zhong and Yao, 2002). Jiu Jiang Tartary is a variety that was bred from mixed local selections using mass selection (Wu et al., 1999).

Mutagenesis and selecting natural mutants: The small flowers of Tartary buckwheat makes artificial crossing difficulty, therefore, mutagenic breeding is a common method in China for Tartary buckwheat breeding (Li et al., 1999). Tang Yu and Zhao Guang are two Tartary buckwheat breeders from Xichang Agricultural College in Sichuan province (Tang and Zhao, 2002). They bred a new Tartary variety, Xi Qiao No. 1 with high yield (average 2,000 kg/ha), high rutin content (1.21%) and wide adaptation, using ^{60}Co-γ radiating seeds. Wei Hei Qiao No. 1, another Tartary variety, was bred by a county agricultural institute using the same radiation method (Mao et al., 2003).

Li Guozhu from Shanxi Agricultural University has studied the effects of ^{60}Co-γ ray in Tartary buckwheat (Li, 2001). The sensitivity to the treatment was genotype dependent. The radiation did not affect the seed germination but inhibited seedling and root development. The treatments significantly affected the height of the plants as well as the numbers of internodes and the first branche.

Selecting natural mutants is a way to increase genetic variability for breeding. For example, a dwarf mutant with high yield and strong growth has been found in a BC_3 generation of common buckwheat (Gao and Liu, 1997). The dwarf character was stable and was passed to the F_1 and F_2 progeny.

Polyploidy: Yu Qiao No. 2 is an autotetraploid variety of common buckwheat that was produced by Gao Li Rong from Shannxi Yulin Agricultural College in the 1980's using colchicine treatment (Lin et al., 1996). Zhao Gang and Tang Yu (1994) obtained an autotetraploid from a diploid variety of Tartary buckwheat. Compared with the original variety, the yield of the autotetraploid increased 10.9%, the rutin content increased more than 50%, and 14 out of 18 amino acids were higher than that of the parental variety. Success in chromosome doubling in Tartary buckwheat also was reported by Lin et al. (1996).

Tissue culture: Improving buckwheat through tissue culture has been attempted in such areas as plant regeneration, anther culture, and protoplast isolation and regeneration (Lin et al., 1996). Kong et al. (1994) regenerated plants from anther culture of common buckwheat. A B5 liquid medium with 0.5 mg/L NAA (naphthalene acetic acid) and 0.5 mg BAP (benzlaminopurine) was optimum after a 48 h pretreatment of the buds. Hao et al. (1994) studied different tissue culture methods in Tartary buckwheat, including callus induction, suspension culture, and a fixed culture. The best medium for callus induction from hypocotyls was MS with auxin at 0.5 mg/l 2,4-D, NAA, and IAA (indole-3-acetic acid) respectively and cytokinin of 0.5 mg/l kinetin and 2 mg/l PUD (phenyl urea derivatives). The fixed culture was found to be better than cell suspension for the production of rutin. Under light conditions, calli produced more rutin than that in the dark. In contrast to calli, the fixed culture produced more rutin in the dark than that under light.

Other methods: Hybridization is a common breeding method in many crops. However, it has not been widely used in buckwheat breeding, especially in Tartary buckwheat breeding. Li et al. (1999) has attempted this method for several years in Tartary buckwheat, however there were no seeds obtained. Except for the structure of the small flower that makes crossing difficulty, the physiological mechanism of fertilization which results in the failure of the crosses is not clear. "Space travel" is a recent breeding technique that has been successfully utilized to breed new varieties of wheat, rice, and vegetables in China. Desirable mutants were produced after the seeds experienced space travel in satellites. This will be one of the new approaches to buckwheat breeding in China in the future (Li et al., 1999).

Genetic studies: Li and Qiao (1997) conducted genetic studies on several traits in Tartary buckwheat using 45 varieties and landraces. The heritability of thousand kernel weight, the number of internodes on the main stem, and plant height were 83.3%, 68.9%, and 61.7%, respectively. The grain weight of a single plant was slightly negatively related to the thousand kernel weight ($r=-0.12$) and slightly positively related to plant height ($r=0.117$).

CULTIVATION

Effects of light and temperature

Buckwheat was found to have three types of reactions to light which were described as sensitive, non-sensitive, and intermediate (Feng et al., 2001). The length of the daylight period affected flower formation (Hao et al., 1995; Hao and Bi 1996; Chai et al., 1998). Short days (less than 14 hours a day) promoted reproduction and 10–12 hours of light per day was optimum. Long days promoted vegetative growth. More than 16 hours of light per day delayed the formation of the flower buds, but flower buds can still be formed under 24 hours of light per day. Within the 6–16 hour day length periods, kernel weight increased as daylight increased, but day length that was greater than 16 hours had a negative effect. Shade conditions and temperature affect the viability of the pollen and seed set (Feng et al., 2001). The most sensitive stage to shade conditions was at the early flower bud formation stage.

The rate of fertilization significantly decreased when temperature was higher than 30°C or lower than 20°C. Based on an experiment of seeding dates (Chai et al., 1995), days from seeding to bud formation was reduced as temperature increased. The effect of temperature on initiation of bud formation was related to the light length. Under a 12 hour day length, temperature had a minimum effect, but under a day length longer than 12 hours or shorter than 8 hours, temperature had a large effect.

Fertilizers

Phosphorous was the most import nutrient to give a yield increase (Chang, 2002) with an improvement of seed set (Tuo et al., 2002). Nitrogen increased kernel weight, while potassium improved both seed set and kernel weight, and seedling stands (Zhong and Yao, 1998; Tuo et al., 2002). Yield increased as the leaf area on the first branch increased when the optimum amount of nitrogen was applied, but yield decreased as the leaf area increased when an excessive amount of nitrogen was applied (Wang, 1998). Farmers usually apply dung (about 20,000 kg/ha) and calcium superphosphate as base nutrients on intermediate and poor fertility soil (Wu et al., 1999).

Seeding time and rate

Buckwheat seeding time varies, depending on the different ecological region: spring buckwheat: late of April–late of June; summer buckwheat: middle of July–early of August; fall buckwheat: late of August–early of September; and winter buckwheat; late of October–middle of November (Xu, 2000). The optimal seeding rates were 1.05 million seeds/ha for common buckwheat and 1.35 million seeds or 87 kg–108 kg/ha for Tartary (Liu J.Y, 2002; Hu, 2003). Seedling density and plant survival had a negative correlation (Hu, 2003). Generally, 1.05 million/ha seedlings for Tartary buckwheat is optimum for plants to use the space efficiently (Wu et al., 1999).

Seed or plant treatments

Soaking seed in liquid fertilizer can nourish the seeds, stimulate germination, improve and strengthen the roots and stems, and increase seed plumpness (Yao et al., 2001). Compared with the non-treatment, after Tartary seeds were soaked in potassium dihydrogen phosphate, potassium permanganate and carbamide, the number of seedlings increased 5%, the leaf color became darker, leaf size enlarged, the plant grew strongly, and yield increased more than 50% due to an increased number of internodes and branches and the weight of single plants and grains. Soaking the seeds in micro-elements (P, Mn, Zn) has also been reported to increase yield (Zhong and Yao, 1997). Prior to seeding, the Yi nationality of Sichuan province are accustomed to coating the seeds with plant ash+calcium superphosphate or plant ash+calcium superphosphate+carbamide to improve Tartary buckwheat yields (Zhao et al., 1999).

Growth regulars, such as BR (Brassinolide) (Tang and Zhao, 2001) and CAU (gibberellins GA4+GA7) (Zhong, et al., 2001), which were sprayed at the seedling or blooming stage increased the chloroplast content and seed set in Tartary buckwheat. Spraying Paclobutrazol, at the stage of bud formation, improved plant lodging resistance due to this chemical functioning in inhibiting growth and reducing plant height, with resulting in yield increases (Zhao and Tang, 2003). Soaking seeds or spraying one-month old seedlings of Tartary buckwheat, with a phenyl urea derivative (PUD), improved root development and photosynthesis (Jia and Zhang, 1997). Under conditions of drought and heat (leaf temperature 38.1–45.3°C), treatments with 10–50 ppm PUD maintained a high content of chloroplasts and rate of photosynthesis. Spraying liquid fertilizers, such as potassium di-hydrogen phosphate (0.1%–0.2%), two or three times at the blooming stage, was one management practice that increased seed set (Wu et al., 1999).

Nine microbial inoculants that function in nitrogen fixation, degeneration of phosphorous and potassium, and resistance to diseases, has been tested in a pot experiment (Shi et al., 2003).The microbial inoculants increased biological and grain yield as well as flavonoid content.

Managements

Management practices to increase buckwheat production include choosing the proper varieties, screening for high quality seeds (selecting plump seeds with the use of muddy water), treating the soil before seeding to kill

pests, preparing a high quality seed bed, applying sufficient fertilizer before seeding, seeding at the proper rate and at the proper time, controlling insects, and harvesting when two third of the seeds mature (Zhang and Yang, 2001). To reduce toxic residues, farmers in many areas now avoid using chemical to treat insects.

UTILIZATION

Food products

China has many buckwheat foods that can be divided into (Lin, 2000; Wang et al., 2002):

1. native foods: For example, buckwheat cake, buckwheat meal, starch jelly, and wines in Yi nationality and cat ear pasta in Shanxi province
2. popular foods:
   a. groats, powder, a thick soup, flour, noodles, instant noodle, bread, dumpling, steamed bun, instant powder, and cereals;
   b. biscuits with reducing sugar, salted multi-layer cake, crispy cake, cake with filling, bread, steam bread, and buckwheat flower candy;
   c. vinegar, wine, tea, curd, powder for drinks, and beverages.
3. functional foods: up to the year 2000, 11 products from 7 research units have been registered for particular health benefits such as powder, tea, noodle, biscuit, capsule.

Other usages

Buckwheat is one of three largest nectar crops in China due to its long flowering period and production of honey containing high quality proteins and glucose (Shi, 2002; Liu et al., 2003).

Buckwheat grain, hulls and straw are used in concentrated feed as well as green silage which has increased the production of milk and eggs in the animal and poultry industries (Shi, 2002; Liu et al., 2003). Buckwheat hull is a high quality pillow filling which keeps biological balance and improves eye health (Liu et al., 2003). Buckwheat straw was also used as a supplement to cultivate mushrooms (Liu et al., 2002).

Since 1990, many health food products made from buckwheat have been developed and released on the market. For example, buckwheat noodles from both common and Tartary buckwheat, Tartary coarse or fine flour for their health components, Tartary tea, vinegar, wine, beer, and capsules of flavonoids (Feng et al., 2001). From among these, Tartary tea and capsules of flavonoids have been exported to Japan and America.

Other products that have been developed (Wang et al., 2002) include buckwheat sausage, healthy vinegar, Tartary rice in Shanxi province; Tartary compounds of powder, particle for their curative effects in Sichuan province; steamed dumpling, steamed bread, starch noodles, pancakes, and noodles in Shannxi province; crisp buckwheat products in Guizhou province; moon cake for mid-autumn festival, wine, and qiao-tuo (similar to bread) in Yunan province; wine, sweet paste, and fermented cake in Inner Mongolia Autonomy. There are also many flavonoid containing products such as toothpaste, flavonoid ointment, sun screen, sun cream, hair condition, bath soap, and natural dye.

General quality of Chinese buckwheat

The quality of buckwheat grain includes three different aspects, i.e. physical, nutrient, and processing quality (Feng et al., 2003). The physical characters of Chinese buckwheat are listed in Table 1.

The quality of Chinese Tartary buckwheat has been analysed based on their collection of local land races (Lin, 2000). The average results of the analysis are in Table 2.

Protein studies

Protein content and composition: The protein content was reported as 10.6–15.5% in Chinese common buckwheat, 11.5–12.0 and 15.7–16.3% in Chinese diploid and tetraploid Tartary buckwheat, 12.7% and 12.2% in wild Tartary and rice Tartary, 10.7% and 9.4 in winged common buckwheat and winged Tartary (Feng et al., 2003) respectively.

Table 3 (Zhang et al., 2003) showed that the albumin and globulin content of buckwheat was close to one half of the total protein content and was higher than that in wheat, indicating the high nutritional value of buckwheat.

Table 1. Physical characters of Chinese buckwheat (Gu, 1999)

Character	Common buckwheat	Tartary buckwheat
Seed length (mm)	4.2–7.2	3.0–7.1
Thousand weight (g)	15–38.8	12–24
Liter weight (g/m^3)	550–600	712–720
Moisture (%)	13	13.5

Table 2. Chinese Tartary buckwheat quality analysis (Lin, 2000).

Character	Average	Highest
protein content %	10.4	11.7
fat acid content %	2.7	3.2
lysine %	0.67	0.96
Vitamin E mg/100 g	2.27	2.49
Vitamin PP mg/100 mg	6.62	8.16
Selenium ppm/100 g	0.20	

Table 3. Protein composition analysis based on 11 buckwheat (BKWT) varieties and lines (Zhang et al., 1998).

Type	albumin	globulin	alb.+glo.	prolamin	glutelin	prola+glu
common bkwt	32.6	16.4	48.9	4.1	14.4	18.5
Tartary bkwt	30.2	16.8	46.9	3.3	15.6	18.9
wheat	14.3	11.8	26.1	33.9	37.3	71.2

The content of prolamin and glutelin in buckwheat was lower than wheat, which is possibly a reason of the lower processing quality in buckwheat than that found in wheat.

Table 4 (Zhang et al., 1998) showed that most of the essential amino acid in buckwheat were higher than those found in wheat, especially for lysine. The essential amino acids and total amino acids in Tartary were higher than in common buckwheat, indicating that the nutritional value of Tartary buckwheat is higher than that of common buckwheat.

Protein accumulation: Grain protein accumulates during the growth period (Chai et al., 1998). The rate of protein accumulation in buckwheat had two peaks. The first peak was three days after flowering, with the highest protein content of 17.32% in common buckwheat and 14.97% in Tartary buckwheat. The second peak was at 23 day after flowering with a protein content of 9.89% in common buckwheat and 9.51% in Tartary buckwheat. The rate of albumin, globulin, prolamin, and glotelin accumulation was similar to the change in grain protein.

Factors that affect proteins: Many factors affect buckwheat protein content and composition (Chai et al., 1998; Zhang et al., 1997a, b, 1998, 2001; Feng et al., 2003). Fertilizer application affects both grain protein content and composition in common buckwheat. Nitrogen plays the most important role in protein, with an increase in protein content and in the content of albumin and globulin with proper levels of application. Phosphorous directly affects yield but it also indirectly affects the protein content as it increases yield that probably dilutes the protein content resulting in an overall decrease in protein percentage (Zhang et al., 1997b).

Either high or low plant density increases protein content and the content of albumin and globulin, but the total yield of protein decreased due to a decrease in grain yield. In an optimum plant density, the protein content of the grain decreased, but total protein yield increased due to the higher yield of the grain (Zhang et al., 1997a).

Rotational crops also affect buckwheat protein. Pea and potato as former crops or a fallow field increased buckwheat yield and protein content more than that of spring wheat and sesame as the preceding crops.

Buckwheat protein varies in different areas in China. Based on the analysis of 1505 Chinese germplasms, Yang and Lu (1990) found that the content of amino acid in North China was higher than that in South China. The amino acid content was highest in Qinghai and Gansu for common buckwheat and in Tibet and Inner Mongolia for Tartary buckwheat.

Table 4. Essential amino acid (AA) content and total amino acid contents based on 11 buckwheat (BKWT) varieties and lines (Zhang et al., 1998) (g/kg protein).

Amino acid (AA)	Common bkwt	Tartary bkwt	Wheat
threonine	37.2	38.7	30
valine	54.5	56.4	42
cystin+methionine	8	10.7	14
isoleucine	41.6	47.5	36
leucine	66.8	72.3	71
phenylalanine+tyrosine	52.5	62.7	45
lysine	58.4	65.1	24
essential AA (g/kg sample)	22.7	30.4	
total AA (g/kg sample)	64.9	86.2	

Starch study

Based on the analysis of 77 samples of common buckwheat and 54 samples of Tartary buckwheat (Li et al., 2001a), the total starch content was 67.66–86.60% in common buckwheat and 69.84–81.35% in Tartary buckwheat.

Buckwheat contains resistant starch which chemically is not a fiber; however, there is an effort to have it declared the same because it acts like soluble fiber in the gastrointestinal tract, thus providing the health benefits of fiber. It cannot be broken down by enzymes in the small intestine but it can be used by the micro-organisms in the large intestine, therefore it is a suitable food for diabetic patients in reducing blood sugar. It also can prevent constipation, hemorrhoids, appendicitis, and intestinal cancer due to its similarity to fiber and the production of butyl acid in the large intestine. Resistant starch was found to be as high as 8.53% in common buckwheat and 10.22% in Tartary buckwheat.

Li et al. (1997) studied the physicochemical properties of buckwheat flour. The swelling power of buckwheat was higher than that of wheat. The pasting characters of buckwheat were a high HPV (hot-paste viscosity) and

CPV (cool-paste viscosity) as well as only a small little effect of NaCl on peak viscosity. Compared with wheat gelatinization, the gel of buckwheat was harder, but WD (the force required to reach gelatinization) and TP (cycle 1 total positive range) were higher.

Processing studies

The development of buckwheat processing in China is leading the world, especially for Tartary buckwheat processing as it is said that Tartary is grown in China, studied in China, and utilized in China (Lin, 2000). Processing varies from small scale, or family size operations, to commercial products produced by machines in large factories, and to health products produced by advanced techniques.

Bread: the evaluation of bread quality in China was based on the rheological constant for flour quality (the rheological constant), flour strength, baking quality (size and hardness), and taste testing (Li et al., 2001). The rheological constant was measured by using a Brabender Farino-graph according to ICC (International Cereal Chemists) standard No. 115. Flour strength was measured using a CHOPIN Alveograph according to ICC standard No. 1. It was shown that the flour produced with a milling rate of 52% was processed easier than that of whole kernel flour with a milling rate 96% (Li et al., 2001). When 10% buckwheat flour was mixed with wheat flour, the bread hardness was similar to bread made from whole wheat flour. When the substituted amount of buckwheat flour for wheat flour was over 20%, the hardness of the bread rapid changed and it quickly lost freshness (Li et al., 2001).

Yogurt: Xu et al. (2001) from the College of Food Sciences in the Southwest Agricultural University have studied the processing of buckwheat yogurt. The best quality of yogurt was made with buckwheat flour: milk=1:2; with a fermenting time of 5 hours, an inoculation rate 3%, and a sugar content of 8%.

Healthy soybean buckwheat milk: Ji (2001) from Jinan University studied the processing the healthy soybean buckwheat milk. The milk was produced with 70% soybean, 30% buckwheat flour, 0.03% sweet leafed chrysanthemum instead of sugar, and 2% milk powder. The content of buckwheat, when it was lower than 20%, affected the health benefits, but when it was over 40%, reduced the flavor and taste.

Buckwheat sausage: The industrialized production of buckwheat sausage has been studied by He et al. (1999) from the Food Department of Shanxi University. The process of production included mixing buckwheat flour with water (1:3)–packaging boxes–cooking at 100°C for 20 min–sealing–cooling down–product.

A thick Tartary buckwheat soup: Xiao (1999) from the Department of Food Processing of Xichang Agricultural College studied the processing of a buckwheat thick soup that was suitable for an aging population or diabetic and high blood pressure patients. The soup was made from 60% pressed and expanded Tartary flour, 15% soybean, 3% peanut, 2% sesame, and 15% sugar to overcome the bitter taste and increase the flavor or it could also omit the sugar for production of a non-sugar type.

Buckwheat sprouts: Buckwheat sprouts are rich in V_{B1}, V_{B2}, Vc, iron, and phosphorouss, especially in rue glucoside that is good for treating diabetes, high blood pressure, and intestinal and stomach diseases, due to its functions in the enforcement and expansion of blood vessel. Jia (2000) from the Changsha Vegetable Institute has developed methods for processing of buckwheat sprouts with optimum conditions, such as temperature (22–25°C), light (dim), and humidity (85%). The sprouts can be harvested in approximately 8 days in summer and 12 days in winter. In the vegetable off season, buckwheat sprouts are produced in the southern part of China between May–July and in the north part of China in the winter or spring periods in greenhouses or under plastic shade (Song and Chen, 1998).

Tartary buckwheat tea: Tartary buckwheat tea is made from the groats. Dehulling of Tartary seeds requires special processing as direct mechanical dehulling is difficult due to the unique seed structure of Tartary buckwheat (Xin et al., 1999). The process developed was: soaking seeds in water for 6 h–removing water–steaming–aging starch at low temperature–drying seeds at 60–70°C with warm air up to 14% moisture–dehulling by machine. This processing can remove more than 50% of the hull. The final step was stir-frying the groats at 180°C for 5–10 min.

Medical studies

Flavonoid: The flour of Tartary buckwheat is rich in flavonoids which may be used to treat type II diabetes (Zhao and Qui, 1997; Zhou et al., 1997; Jiang et al., 2001). Both animal experiments and clinical observations were conducted in the Shanghai Disease Prevention Center and at the Medical University of Shanghai. Both normal and high blood sugar rats were fed a diet containing 0% (control), 2%, 20%, and 60% Tartary buckwheat flour, respectively. After 7 days, the rats were given 2.5 g/kg body weight of glucose to test their sugar tolerance. The blood sugar in the empty stomach (FBG) and the rate of sugar tolerance (GT) of the rats were measured at 0, 0.5 h and at 2 h. The results showed that the blood sugar level was significantly lower in the treated groups as compared to the control groups. In a clinical experiment, one half of 64 diabetic type II patients were given 100 g of Tartary flour once a day in their diet for 5 weeks. In comparison to the initial rating and to the non-treated group, the FBG rate, TG (blood serum tri-

glyceride) in the treated group were significantly decreased, but the decreased TC (total blood serum cholesterol) rate did not reached the significant level. The symptoms in the treated group, such as excessive over drinking and eating, excessive urination, and numbed hands and feet were obviously improved.

Composition of Tartary protein complex and the role on resistance to aging: Zhang et al. (2000a) from Shanxi University studied the composition of the Tartary protein complex (TBPC) and its roles on resistance to aging. TBPC which was extracted by NaOH contained 63.4% proteins and additional fatty acid carbohydrates, ash, fiber, and water. After the experimental rats were fed TBPC for 20 days, the enzyme activities (SOD, CAT, and GSH-Px) in the blood, liver, and heart was increased by 13.4%–23.2%, while the content of malonaldehyde (MDA), a peroxidated product, was reduced, indicating that TBPC has a role in resistance to aging.

Peroxidase: Peroxidase is a type of enzyme in organisms that functions in repairing damage in the organism caused by peroxides; therefore they are widely used for labeling enzymes and immunological studies. Zhang et al. (2000b) from Shanxi University found a superoxide dismutase (SOD) from Tartary buckwheat leaves in 1993 and purified and characterized a new peroxidase from Tartary buckwheat bran later. These new findings will be useful for Tartary buckwheat utilization in the medical area and also as a functional food.

Medical products

Since 1984, the Beijing Grain Science Research Institute has set up a project to study flavonoids in Tartary buckwheat and cooperated with other research units in agriculture, grain, medicine, and food sciences (Zhao and Qu, 1997; Lang, 1997; He, 1998). The extraction of biological flavonoids mainly consists of quencetin, rutin, kaenpferol, and morin. Many medical products were made from this extract, with supplementation of a few Chinese herbs, as capsules, powder, ointment, toothpaste, and gum for internal for external uses. In clinical observations, they were efficient for the treatment of various diseases, such as diabetes, high blood cholesterol, stomach diseases, tumors of the digestive tract, liver cancer, vascular diseases, herpes zoster, bedsore, scald, wound infections, pharyngitis, gingivitis, mouth ulcer, and bad breath. Buckwheat flavonoids have also been used for coloring in costumes.

A capsule of *San Xiao Ling* was made from Tartary buckwheat produced by the White Agricultural Center in Shanxi province (Lin, 2000). It was used for treating diabetic type II patients. This product did not affect the blood sugar of normal rats, but it significantly reduced the blood sugar and increased the sugar tolerance in rats with alloxan diabetes. The fasting blood sugar of the diabetes patients was reduced and symptoms, such as excessive drinking and eating, were improved after they took this product for one month.

In addition, *168 Jiang Tang Bao Xian* was made from Tartary buckwheat by Xian Lesi Health Products Ltd. also for treating diabetes (Wang et al., 2002). *Jin Qiao Mei Pian* was made from *F. cymosum* by the Chinese Academy of Science for treatment of lung cancer and breathing diseases (Wang et al., 2002).

OTHER STUDIES

Wang Rui from the Shanxi Agricultural Academy and Li Chang An from Shanxi University studied insect pollinators on common buckwheat (Wang and Li, 1998). Bees, from the family *Apoidea* in the *Hymenoptera* order, are the main pollinators (65%) which include the main species of *Apis Cerana+A. mellifera* (35%) and *Bombus* sp.+*Andrena* sp.+*Omsia* sp.+*Megachile* sp. (25%). Other pollinators included the *Syrphidae* family (main species: *S. cripta*) in *Diptera* order (20%) and butterflies+moths+beetles (10%). Most of the labeled insects pollinated plants within 1,000 m. The flying distance of the feeding bees from the hives to the buckwheat field was approximate 2,000 m. One hectare of buckwheat can produce 60–112 kg honey. One bee can carry 10,000 pollen grains and collect from 500 flowers on one trip.

Gallerucida bifasciata is a type of leaf beetle that can destroy up to 100% of the buckwheat leaves, causing 5%–10% yield loss (Li and Li, 2003). This insect produces only one generation each year and the young beetles are hatched from over-wintering adults, resulting in damage to the buckwheat. Proper field management combined with chemicals can control the damage.

Spica parallelangula is a type of moth that seriously damages buckwheat plants (more than 50% plant) in the southwest of China (Li et al., 1996) and sometimes even causes total yield loss. This insect produces only one generation each year. The nymph hibernates for more than seven months. Low temperature (12–16°C) and high humidity (80%) can result in severe damage.

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The 9th International Symposium on Buckwheat

by Mr. Zdeněk Stehno
Active Chairman of the organizing committee
Research Institute of Crop Production
Drnovska 507, 161 06 Prague 6, Czech Republic

Three years after the last symposium on buckwheat was held in Korea in 2001, the 9th International Symposium on Buckwheat was organised on 18–22 August 2004 in the Congress Centre of the Agricultural University in Prague, Czech Republic. The symposium was prepared by the organising committee consisting mainly of personnel from the Research Institute of Crop Production Prague under the Auspices of the International Buckwheat Research Association (IBRA) and Ministry of Agriculture of the Czech Republic.

Keynote presentations were devoted to the development and importance of IBRA (I. Kreft), the origin of cultivated buckwheat (O. Ohnishi), advances in overcoming breeding barriers in buckwheat (T. Adachi), the present state and future prospects for buckwheat (C. Campbell) and the status of buckwheat culture and use in the Czech Republic (J. Petr et al.).

The section 'Biotechnology and Physiology' was aimed at buckwheat DNA analyses and utilization of specific genes and methods of tissue culture for special purposes. Physiological aspects of stress conditions and other environmental factors were described and discussed. The phases of buckwheat development as influenced by day length and growing in northern localities were described for *Fagopyrum esculentum* as well as for *F. tataricum.*

In the section "Genetics, Genetic Resources and Breeding", attention was paid to buckwheat genetic resources which are available in different countries and regions and to the conservation of buckwheat diversity in general. The genetics of the crop that represents the basis for buckwheat breeding was mentioned in many presentations. Inheritance of certain traits, such as homostyly, branching, determinant flowering, rutin content, seed protein polymorphism etc. was evaluated and presented. Research using new breeding methods and supported selection techniques such as molecular markers, micropropagation, interspecific hybridization, polyploidization and others were reported.

Different techniques of buckwheat cultivation were presented in the section "Cultivation and Plant Nutrition". The impact of different fertilisers and forms of application was evaluated and described frequently in this section. In addition, disease occurrence, phytopathological monitoring and plant-pathogen relations were reviewed. As well the yield obtained under different techniques of cultivation and its components were analysed.

The final section was devoted to "Food Processing, Health and Functional Food". This part of the symposium included contributions on the composition of buckwheat seed as a final product of its production and as raw material for further processing. Flavonoids in general and especially rutin were studied in detail. The possible substitution of buckwheat for wheat flour was also evaluated. Aspects of buckwheat allergy or asthma were described in a few presentations. Long term tradition of buckwheat consumption and traditional buckwheat dishes were also described and remembered.

The symposium consisted of two main parts: oral presentations and a poster session.

The programme was completed with a section devoted to buckwheat utilization in the system of organic farming. This section was held in the Czech language and the presentations of foreign speakers were translated.

Over one hundred specialists from abroad and approximately twenty five participants from the Czech Republic took part in the symposium (Fig. 1). In addition, a group of 16 Japanese tourists also attended the opening ceremony of the symposium.

In the final plenary IBRA meeting in which all participants took part, Dr. Anna Michalova, CSc. from the Research Institute of Crop Production, Prague, Czech Republic was elected President. Prof. Cheol Ho Park was appointed as Acting President until Dr. Mikalova can assume her duties. The Tenth International Symposium on Buckwheat will be held in Yangling, Shaanxi, China in 2007 and will be organized by Dr. Chai Yan.

The symposium was completed with a one day scientific excursion, organised as a part of a post symposium tour, with stops at buckwheat fields (Fig. 2), buckwheat processing firms and at a traditional buckwheat mill. Optional post symposium tour II organized by Prof. I. Kreft was a week-long journey from Czech to Italy via Austria and Slovenia with stops at buckwheat fields (Fig. 3) and many old cities in Central Europe.

Technical note:

Proceedings of the symposium are still available from the organising committee at the following ddress: Research Institute of Crop Production, Gene Bank (Zdenek Stehno), Drnovska 507, 161 06 Prague 6–Ruzyne, Czech Republic or from their e-mail address: stehno@vurv.cz.

Fig. 1. Participants of 9th International Symposium on Buckwheat at Prague, Czech Republic.

Fig. 2. Bohemian buckwheat field near Orlicke Hory (photographed by Dr. K. Ikeda).

Fig. 3. Buckwheat fields near Maribor, Slovenia (photographed by Dr. K. Ikeda).

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Nakajo 1, Mozume-cho, Muko 617–0001, Japan
E-mail address: ohnishi@mbox.kudpc.kyoto-u.ac.jp

Manuscript should be typed double-spaced throughout on one side of DIN A4 paper (21×29 cm or 8.5×11 inch) with sufficiently wide margins (2.5–3 cm). All pages, including the tables, legends and references, should be numbered consecutively. In order to expedite publication and to reduce costs, submitting the manuscript on diskette is recommended (but this is not a necessary condition for publication). On submitting a diskette, specify the type of computer and word processing software by which the authors processed their manuscript file.

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