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CONTENTS

Fagopyrum 20: 1–6 (2003)

Buckwheat Crop Improvement

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ABSTRACT

Crop improvement programs on common buckwheat have been documented for over one hundred years although yield and other desirable improvement characteristics have not kept pace with other larger domesticated crops. Increased economic importance that is being placed on Tartary buckwheat products due to their increased health benefits should see increased efforts to improve this species. The development of self-pollinating buckwheat has received increased efforts over the past decade and appears to be an area that can be utilized for the improvement of underutilized common buckwheat.

INTRODUCTION

Buckwheat, which must be considered an under utilized crop, is of significant economic importance in only several countries of the world although it is produced in many countries and on every continent. The two species which are of the highest economic importance are *Fagopyrum esculentum*; generally called Common Buckwheat, which is the main species under cultivation and production for international trade; and *F. tataricum*, or Tartary Buckwheat which is produced in many areas of the world but is second in economic importance.

Although the genus *Fagopyrum* contains at least eighteen species of buckwheat only two are utilized as food or feed and one other is used as livestock fodder. However, in comparison to the cereal crops, the seed yield of *F. esculentum* is often relatively low and unstable. Tartary buckwheat, on the other hand, has a tightly adhering husk that is hard to remove, a low groat content and contains a bitter component that limits it's use as a food although there has been recent attention being given this species due to its having compounds that are beneficial to health. Wild buckwheat *F. cymosum*, found as a diploid in China and mainly found as a tetraploid in other countries, is also utilized in some areas as a green vegetable or as cattle forage.

Common buckwheat is a self-incompatible species with outcrossing characteristics and this therefore dictates the breeding patterns that are most commonly used on it. All lines that are being developed must be kept in isolation, either spatial or in cages, from each other which severely limits the number of lines which may be developed annually.

Tartary buckwheat crop improvement programs can be handled in the same manner as other self-pollinating crops. It is thus much easier to generate and maintain the large numbers of segregating progeny and advanced lines that are required in any plant improvement program researching this species. Plants of Tartary buckwheat are also generally recognized as being hardier and having more resistance to drought and frost as well as to other environmental stresses.

The perennial species, *F. cymosum*, has rhizomes and differs from Tartary and common buckwheat in its shoots, branching and racemes. It can be found in China and through out the Himalayan regions of Butan, Nepal and India. There it is utilized as a fodder plant for livestock grazing.

During the past decade more wild relatives of common and Tartary buckwheat have been found and they have become increasingly more important in plant improvement programs. This has in part been due to the finding and classification of the new species and a much better understanding of the area of origin of buckwheat species and possible progenitor species of these economically important crop plants. It has also been due to the increased emphasis that is being placed on inter-specific hybridization by many of the buckwheat improvement programs.

History of Buckwheat Improvement Programs: Common buckwheat *Fagopyrum esculentum* Moech

Crop improvement programs have been active on common buckwheat for a very long time. As an example the variety Bogatyr was developed at the Shatilovskaya Research Station with the development being done from 1901 to 1909. (Fesenko, 1990). In Japan the variety Hashigamiwase was bred in 1919 and the variety Botan Soba was released in 1930 with Shinano No. 1 being released in 1944. In 1968 E.S. Alekseeva bred the variety Victoria. (Fesenko, 1990). Some of these original varieties are still being grown to a limited extend at the present time. This clearly shows that progress on crop improvement in common buckwheat has been slow due to its' out-crossing nature and also due to limited variation that appears to be present in this species.

Several crop improvement programs started to breed for large seeded buckwheat in the 1960's. Before this almost all varieties had a grain weight of 20–22 g/1000 seeds. The first large seeded varieties produced were Shatilovskaya 5 in 1967 with a seed weight of 28 g/1000 seeds (Fesenko, 1990). The variety Mancan was produced in Canada in 1974 and the variety Kitawase was released in Japan in 1989. Larger seeded varieties have since been produced with Russia releasing Krasnosteetskaya with a grain weight of 33 g/1000 seeds and Canada releasing Koto with a grain weight of 36 g/1000 seeds.

Tetraploid varieties began to be produced in the USSR in the 1940s (Fesenko, 1990). Some of these varieties were Bolshevik 4, Iskra, and Minchanka. Tetraploid varieties, such as Miyazakiootsubu was released in Japan in 1972 followed by Shinshuoosoba in 1974. These varieties had increased lodging resistance, however, problems with decreased fertility and increased hull thickness has caused most crop improvement programs to abandon this method of crop improvement. Shorter plant types have been considered in the development of lodging resistance. In the USSR I.V. Aleekseeva using buckwheat starting material of different stem lengths developed plants of intermediate type with a compact stem and standard height. (Alekseeva, 1994).

Breeding for mutational characteristics has also been done by many crop improvement programs. The determinant type of plant habit gave limited growth, lodging resistance and a narrow leaf and was therefore was suited to regions with a short hot summer. The determinant character was first reported in the USSR 1940 but breeding did not begin until 1954 in this country. The variety Sumchanka is an example of this type of plant habit. The determinant type has also received attention in crop improvement programs in Japan. Self-compatible common buckwheat has also received attention. They have been developed from natural mutations and by ion exposure. The stable self-compatible lines produced by Nomura et al. (2002) were all thrum plants. Inbreeding depression occurred in the selfed lines.

Although buckwheat has been described as having different day length requirements in many countries some crop improvement programs attempt to breed for day neutral plants. However, short season crops are desired in many areas for their utilization as a second crop or under conditions of heat and low moisture. The variety Skorospelaya, which is an ultra quick ripening variety, was developed in the USSR for such a purpose.

Unstable seed yields in common buckwheat have been the major limiting factor for this crop. It has been shown that very high levels of pollination occur (Morris, 1952). The rate of fertilization is also high, although increased fertility has been reported by utilizing a modified recurrent selection procedure (Inoue, 2002).

Other aspects of common buckwheat have also been addressed. Expansion of first true leaf, plant height, branching, time of flowering, number of seeds per plant, yield, and harvest index was reported by Mather (1991).

***Tartary Buckwheat* (*Fagopyrum tataricum* Gaertn.)**

Plants of *F. tataricum* are usually less husky in growth than those of *F. esculentum* although large types are also present. They are more branched and the leaves are more arrow shaped. The flowers are smaller, have inconspicuous greenish-white sepals and do not appear to be attractive to insects. The flowers are homomorphic, self-fertile and are cleistogamous, with pollination occurring before the flower opens. The first buckwheat variety released in Canada was the Tartary buckwheat variety Welsford developed in 1946.

As Tartary buckwheat is self-pollinating much of the work on it has been by single plant selections. This has been successful in increasing components, such as rutin, however, only a few crop improvement programs worldwide are working on Tartary buckwheat. Of the two cultivated species, Tartary buckwheat was found to have fewer alleles per locus (1.40 vs 1.59) and fewer alleles per polymorphic locus (2.00 vs 2.38) than common buckwheat (Huh et al., 2001).

The finding of 'rice' Tartary buckwheat which has a non-adhering hull and therefore dehulls very readily, has been reported in Nepal, Bhutan and southern China. This allows the use of it as a rice replacement in the staple diet in these production areas. It is surprising that this interesting trait, although reported as desirable, has not resulted in increased production of this type of Tartary buckwheat. Perhaps this has been due to lack of crop improvement efforts that must address yield as well as dehulling capabilities. Crosses between Rice buckwheat and normal Tartary buckwheat have been extremely difficult to accomplish (personal data) which may account for the lack of progress in this area.

The improvement of the species *F. tataricum*, although secondary in many breeding programs, is of major importance in the areas of the world that rely on this crop. These areas are mainly the mountainous regions above 2500 m in altitude that have a danger of frost damage to the crop. As pointed out by Ohnishi (1995) a putative progenitor species that has a self-incompatible pollination mechanism has still not been found. The finding of such a progenitor species would benefit any crop improvement program working on Tartary buckwheat, or on any program desiring to transfer traits into other economically important species.

F. tataricum has long been considered as a parent in inter-specific crosses due to it having many desirable traits such as higher seed yields, self-pollination ability, frost resistance and overall plant vigour. These traits, however, are not only present in this species but in other species as well. The finding, identification and classification of additional buckwheat species by Ohnishi (1990) has now opened the door to increased inter-specific opportunities in buckwheat.

Other species

As the species *Fagopyrum cymosum* was in the past generally believed to be the progenitor of common and Tartary buckwheat the first attempts on inter-specific hybridization was between it and *F. tataricum* (Krotov, 1975). This was followed by Ujihara et al. (1990) who crossed it with *F. esculentum*. Both of these crosses were accomplished at the tetraploid level as fertile progeny have not yet been possible at the diploid level. The development of the inter-specific hybrid at a diploid level between *F. tataricum* and *F. esculentum* has been accomplished by Bjorkman and Samimy (Samimy et al., 1996) and by Hirose (Hirose et al., 1995). The finding by Ohnishi of *F. esculentum* ssp. *ancestrale* and the closely related species *F. homotropicum* has increased interest in the development of inter-specific hybrids at the diploid level utilizing these species (Ohnishi, 1991).

The progenitor species *F. esculentum* ssp. *ancestrale* was found on Oct 29, 1990 at Lijiang valley, Yunnan province. In the words of its finder "Finally I would like to mention that my discovery may provide good hope for the utilization of the wild ancestor as a genetic resource for future buckwheat breeding" it issued in a new era of crop improvement. These words have now proven to be true in several crop improvement programs utilizing wild species. This has been accomplished by Campbell (1995) and by Hirose (1995) and by Woo et al. (2001) with the development of hybrids between *F. esculentum* and *F. homotropicum*. The main interest in breeding programs has been the development of methodology that will allow the movement of specific characteristics from related species into common buckwheat. The first reported successful transfer has been the movement of the self-pollinating characteristic from *F. homotropicum* into *F. esculentum* as reported by Campbell (1995). *F. esculentum* ssp. *ancestralis* has fragility of premature seeds, strong seed dormancy, longer growth period and branching at lower nodes than common buckwheat. However, these traits can readily be eliminated or changed through backcrossing with *Fagopyrum esculentum*.

A large number of new wild species have been reported over the last decade (Ohnishi, 1991, 1995, 1998; Ohnisi and Yasui, 1998; Chen, 1998b). Three new species were suggested by Chen (1998) from collections in Tibet, Sichuan, Guizhou and Yunnan (Chen, 1998b). The finding of the progenitor species for common buckwheat was reported by Ohnishi (1991) who has published many subsequent reports. An example is reporting on the morphological characteristics of one sub species *Fagopyrum esculentum* ssp. *ancestrale* and four new species *F. homotropicum, F. pleiormosum, F. capillatum* and *F. callianthum* in the genus *Fagopyrum* by Ohnishi (1998). This area is still one of great interest as the use of the wild species as genetic material in crop improvement programs increases.

Inter-specific crosses

Many attempts have been made to develop inter-specific hybrids in buckwheat using *Fagopyrum cymosum* as one of the parents. A successful inter-specific hybrid between *Fagopyrum tataricum* and *Fagopyrum cymosum,* in the tetraploid state, was reported by Krotov (1975) and named *F. giganteum*. The next successful cross between *Fagopyrum esculentum* and *Fagopyrum cymosum* was accomplished by Ujihara et al. (1990; Hirose et al., 1993), again at the tetraploid level. This was accomplished using ovule culture and further back-crossing of the hybrids with common buckwheat have resulted in fertilized ovules which were embryo rescued, however, the resulting plants were sterile. This hybrid was again produced by Suvorova et al. (1994) through the use of ovule rescue. Hybrid plants were obtained. The interspecific hybrids possessed a phenotype close to *Fagopyrum cymosum* and were characterized by complete sterility in all pollination types.

Conventional breeding techniques have not been successful in crossing common and Tartary buckwheat (Morris, 1952; Ruszkowski, 1980; Adachi et al., 1989). A number of studies on the development of inter-specific hybrids have attempted to overcome cross incompatibility by using embryo or ovule culture systems (Hu and Wang, 1986). Successful hybridization of Tartary buckwheat with common buckwheat at the diploid level has been accomplished by Cyrus Samimy and Thomas Bjorkman of Cornell University (Samimy et al., 1996). These plants grew in the greenhouse but were sterile. Hirose (Hirose et al., 1995) developed the same and reciprocal crosses of these two species.

Campbell (1995) reported on the first successful inter-specific hybridization of buckwheat, at the diploid level, in which the progeny are fertile and in which further back-crosses to common buckwheat have been carried out. Using conventional pollination at the diploid level, successfully hybridized of *Fagopyrum esculentum* Moench. and *F. homotropicum* Ohnishi (both diploid, 2n=16) was accomplished. This hybridization produced fertile progeny that have been back-crossed to *F. esculentum* to transfer the desirable traits of *F. homotropicum* to common buckwheat. As *F. homotropicum* has a self-pollinating system this trait were considered to be the most desirable to transfer into common buckwheat. The *F. homotropicum* parent, unfortunately has severe seed shattering due to the development of an abscision layer. This undesirable character, however, can be readily eliminated after hybridization. As *F. homotropicum* occurs as both a diploid and tetraploid forms both have been utilized in breeding programs (Wang et al., 2001).

The successful inter-specific hybridization of *Fagopyrum esculentum* and *F. homotropicum* at the diploid level opened a new area for the improvement of common buckwheat. Traits such as self-pollination and seed and plant characteristics have now been transferred to common buckwheat. The species *F. homotropicum* is a self-pollinating species that sets all of its seeds before flower opening, in the same manner as Tartary buckwheat. As common buckwheat has a seed abortion problem so severe that often only approximately 10 to 12% of the flowers produce mature seeds the transfer of the self-pollinating mechanism into common buckwheat allows for increased yield. However, the high number of flowers produced by *Fagopyrum esculentum* must be drastically reduced to allow for the nutrients now being utilized for their growth to be diverted into filling the increased number of seeds being formed. Kim et al. (2002) found that in crosses with varieties that the highest seed set of the hybrids was 44.5%.

The development of inter-specific hybrids between *F. homotropicum* and *F. tataricum* is of interest in a number of crop improvement programs. If successful these would allow the transfer of characteristics between these two species. However, all crosses attempted to date have been sterile. One possible reason for this may be chromosome size. Genome size of the various species of the genus Fagopyrum were determined by flow cytometry using propidium iodide (PI) as flurochrome. The C-values varied widely, from 0.55 for *F. tataricum* to 1.92 for *F. urophyllum*. *F. esculentum* was at 1.39 or mid way among the species (Nagano et al., 2000).

The use of the hybrid resulting from crossing *Fagopyrum esculentum* with *F. homotropicum* as a bridge species for the cross of *F. esculentum* by *F. tataricum* has shown some promise with improved fertility of the pollen (Wang et al., 2002). The progeny were sterile, however, the use of the *F. esculentum/F. homotropicum* hybrid as a bridge species increased the pollen viability to approximately 22% and might be a possible means of transferring genetic material between the two species. If genetic material can be transferred between the two species it would allow not only for the improvement of common buckwheat but the improvement of Tartary buckwheat as well, an area that is of great interest in the Tartary buckwheat producing areas of the world. These areas include China, Bhutan, Nepal, Northern India, and Northern Pakistan as well as other production areas.

Fertile *Fagopyrum cymosum* and *F. tataricum* hybrids as well as *F. cymosum* by *F. esculentum* hybrids have been produced at the tetraploid level. *F. esculentum by F. homotropicum* hybrids have proven to be fertile at the diploid level. *F. esculentum* by *F. tataricum* hybrids have been developed at the tetraploid level and are presently being attempted at the diploid level. Fourteen interspecific hybrids were obtained through ovule culture between cultivated tetraploid common buckwheat (*Fagopyrum esculentum*, 2n=32) and the wild perennial species (Fagopyrum cymosum, 2n=32) originating in Nepal (Hirose et al., 1993). Five interspecific hybrids were produced between *Fagopyrum cymosum* and other species *(F. esculentum, F. tatarticum, F. homotropicum F. pilus* and self pollinating buckwheat through embryo rescue (Woo et al., 1999). However, to date all progeny have proven to be sterile. Reports have also been made of wide hybridization attempts within the genus *Fagopyrum* (Chen, 1998a) although no reports have been made of fertile progeny.

Regeneration of plant parts has also received a large amount of attention as a possible means of increasing valuable genetic material. Shoot regeneration from mature cotyledons was tested in 33 diploid and 4 tetraploid varieties belonging to the Slovenian buckwheat germplasm collection. They demonstrated that both explant viability and shoot regeneration are strongly influenced by genotype and that a high variability can be found for these traits among and within buckwheat populations. "A high frequency of plant regeneration is one of the essential prerequisites for the application of tissue culture in crop improvement" (Luthar and Marchetti, 1994).

Plant genetic resources

There have been several major efforts to collect and preserve buckwheat germplasm. The two largest have been the USSR and China. In China collections and evaluations were done after 1978 and have resulted in more than 3,000 accessions (Yang, 1995). In 1989 to 1990 China had a nation wide regional test on superior varieties of buckwheat, China's buckwheat production and variety evolution. They looked at the ecological type and adaptation of buckwheat varieties and land races (Rufa et al., 1991). After the breakup of the Soviet Union, Ukraine also has maintained a large collection of over 2,200 accessions of buckwheat (Bochkareva, 1995).

New areas of work

The use of DNA techniques is rapidly expanding in crop improvement programs. Analysis of a limited number of individuals of different cultivars and populations of *Fagopyrum esculentum* Moench. and populations of *Fagopyrum tataricum* Gaertn. using three primers, showed high polymorphism within cultivars and populations and three cultivar specific RAPD were detected. RAPD analysis seems very promising in studying genetic variability in buckwheat (Javornik and Kump, 1993).

The development of haploids for utilization in crop improvement programs is presently receiving attention. Anthers of three Polish bred common buckwheat cultivars were cultivated in vitro. The most prevalent class of regenerates was tetraploid regardless of cultivar. No haploids were found among the regenerates derived from the diploid cultivars likewise the tetraploid cultivar yielded no diploid regenerates (Berbec and Doroszewska, 1999).

Mesophyll protoplasts of *Fagopyrum esculentum* have also been fused by PEG-mediated fusion with hypocotyl protoplasts of *F. tataricum* serving as the hauler. A simple two step preselection method using the intolerance of mesophyll protoplasts to the fusion procedure and the appearance of *F. tataricum* calli could be established. The hybrid nature of the calli could be confirmed by RFLP analysis. (Javornik and Kump, 1993).

Another area receiving attention is for the identification of allergenic proteins in buckwheat and their removal. The major allergenic protein with a molecular mass of 22 kDa was identified and sequenced. It was their hope that the nucleotide information can be effectively used to develop hypoallergenic buckwheat (Nair et al., 1999).

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Fagopyrum 20: 7-11 (2003)

Rutin in buckwheat — Protection of plants and its importance for the production of functional food

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ABSTRACT

The amount of ultraviolet (UV)-B absorbing compounds in buckwheat is related to UV-B radiation. The increased terminal electron transport system (ETS) activity of mitochondria of plants, exposed to UV radiation, is suggested to reflect the energetic cost of generating the internal mechanisms for photoprotection. Rutin, quercetin, quercitrin and other flavonoids are secondary plant metabolites and are synthesized in higher plants in order to protect the plants against UV radiation, diseases and predators. Buckwheat is the only field crop that contains rutin and due to its high nutritional value is considered as an important source for functional food. The majority of the rutin is synthesized in the herb, especially in the inflorescence. The seeds contain less flavonoids, which are localized mostly in the testa and cotyledons. Green buckwheat tea has became used more since it is known for its content of rutin and its beneficial effects on cardiovascular disease, reducing risk of arteriosclerosis and also for its antioxidative effect.

INTRODUCTION

Beneficial effect of vegetables and fruits in preventive nutrition is strongly connected to the content of flavonoids (Williamson et al., 2000; Kim et al., 2001). The basic polyphenol molecule can be coupled to different sugar molecules (Hollman et al., 1996). Such connections can diminish their biological effects, however, the complex molecule may be more soluble and more easily transported throughout the body. Polyphenols may influence gene expression, inhibit enzymes or can be selectively bound to receptors in the cells (Williamson et al., 2000; Gee, 2001; Johnson, 2001; Carlsen et al., 2001; Myhrstad et al., 2001).

IMPORTANCE OF RUTIN FOR THE PROTECTION OF BUCKWHEAT PLANT

Many biological and physiological processes in plants can be affected by UV-B radiation (Häder et al., 1998; Rozema et al., 1997; Björn, 1999). Species and populations originating from naturally high UV-B locations, i.e. from high mountains and/or low latitudes, are less sensitive to UV-B radiation than plants from other locations and which are exposed to weaker UV-B radiation (Sullivan et al., 1992; Xiong et al., 1996; Villafaòe et al., 1999). Eastern Tibet and adjacent areas of Sichuan and Yunnan are the probable sites of the origin of cultivated common and Tartary buckwheat (Ohnishi, 2002, 2003). The altitude of the villages and towns in Eastern Tibet is in the range of 2500 to 3500 m above sea level (Ohnishi and Konishi, 2001). The buckwheat plant originated in locations exposed to high UV-B radiation because of the high altitude and relatively low latitude. The ability to synthesize rutin and related compounds is thus an evolutionary plant response to the extreme growing conditions. For photosynthetic organisms that depend on solar radiation as the primary source of energy in their natural environment it is very important to be protected against direct and indirect influences of UV-B (Hessen et al., 1995). One of the key responses of plants to UV-B is the synthesis of UV-B absorbing compounds, such as rutin, quercetin and quercitrin, which protect the cells by preventing UV radiation from reaching and damaging the vital molecules, such as nucleic acids, and especially DNA (Häder et al., 1998; Björn, 1999; Gaberščik et al., 2002a; Germ et al., 2002; Villafaòe et al., 1999).

Literature reviews show that the production of UV-B absorbing compounds in primary producers in high elevation ecosystems was a response to the higher flux of UV-B. The frequently observed correlation between UV-B radiation and the concentration of UV-B absorbing compounds in many primary producers shows that these substances protect vulnerable targets in organisms (Sommaruga and Garcia-Pichel, 1999; Gaberščik et al., 2002a; Germ et al., 2002; Winkel-Shirley, 2002).

The synthesis of secondary substances occurs in other plants as well as in buckwheat. They are energetically costly and are produced if the damage due to UV-B is greater than metabolic costs of production (Gaberščik et al., 2002b). The terminal electron transport system (ETS) activity of mitochondria is possible to be scored as described by Packard (1971). ETS activity is a measure of the metabolic potential of organisms. It has already been reported, that ETS activity increased under enhanced UV-B radiation (Ferreyra et al., 1997; Scott et al., 1999; Gaberščik et al., 2002a; Germ et al., 2002). This can be explained by the increased need for energy for the mechanisms involved in repairing damage and for photoprotection, i.e. production of UV-B absorbing compounds. An organism might be able to tolerate UV damage, but if the energetic costs of protection and recovery are too great, reproductive success could be compromised (Cullen and Neale, 1994; Karentz et al., 1994). ETS research is going on as well in buckwheat (unpublished results).

Along with other bitter and astringent substances, for example tannin (Luthar, 1992), rutin may protect also buckwheat plant against grazing by wild or domestic animals and insects. However, these substances may additionally defend buckwheat plant and seeds against fungal and bacterial attack.

SYNTHESIS AND ALLOCATION OF RUTIN AND RELATED COMPOUNDS IN BUCKWHEAT

Kitabayashi et al. (1995) studied varietal differences and heritability of rutin content in buckwheat. Michalova et al. (1998) reported on differences in the rutin content among buckwheat gene bank samples. Fujita et al. (2003) established varietal differences of antioxidant activity in tartary buckwheat. According to their results, it is obvious that as the spread of cultivars from the site of origin to regions with warm and humid climatic conditions, their antioxidant activity and chemical defense against bacteria, fungi and viruses, produced by the same substances became important. Lee et al. (2001a, b) investigated the interaction of the genetic component (buckwheat varieties) and the spectral composition of visible light on the rutin content in buckwheat. Rutin may be up to 300-fold more highly concentrated in groats of Tartary buckwheat than in groats of common buckwheat (Steadman et al., 2001). According to Kreft et al. (2002) the synthesis of rutin and other polyphenolic compounds may be influenced by ultraviolet rays. Modest amount of UV-B radiation may stimulate the synthesis of rutin, however, buckwheat plants may be damaged by higher doses (Kreft et al., 2002; Gao et al., 2002). The tissue-specific distribution of rutin and rutin-catabolizing enzymes was studied in buckwheat leaves by Zhanaeva (1998). The highest amounts of rutin was contained in the upper epidermis and the glycosidase was the most active in the lower epidermis.

Wagenbreth et al. (1996) found variable rutin content (2% to 9%, d.m.b.—dry matter basis) in leaves of different buckwheat cultivars. In leaves situated higher on the plant there was more rutin than in the lower ones. They found the highest rutin content in the inflorescences (up to 12%, d.m.b.).

According to Oomah and Mazza (1996) the site of production has an important influence on the content of rutin and other flavonoids in buckwheat seeds. As reported by Schneider et al. (1996), more rutin is synthesised at higher temperature (24.5°C daytime, and 18°C at night) in comparison to lower temperature (18°C daytime, and 12°C at night).

Qian et al. (1999) studied antioxidants in five cultivars of buckwheat. Differences in the content of rutin (3.8 to 10.1 mg/100 g of flour) and in the content of total antioxidants were established among the cultivars. According to Ohsawa and Tsutsumi (1995) there are considerable differences in rutin content among buckwheat cultivars; later flowering cultivars may have a higher rutin content. Genetically based differences in rutin content may also be found among individual plants (Kreft et al., 1999).

According to Noguchi and Mori (1969), Yasuda et al. (1992), Morishita et al. (1998), Sindoh et al. (2001), and Suzuki et al. (2002) rutin may be degraded by flavonol 3-glucosidase, a rutin-degrading enzyme, present in buckwheat plants (for example in the testa).

Dietrych-Szostak and Oleszek (1999) investigated the influence of food processing on the flavonoid content. Park et al. (2000) studied rutin content in different buckwheat species and in different products (tea from buckwheat flowers, green flour from leaves, popped buckwheat groats).

BUCKWHEAT RUTIN, QUERCETIN AND QUERCITRIN IN THE HUMAN DIET

The health effects of rutin and related flavonoids were established by Griffith et al. (1955). The effects of rutin from buckwheat-herb tea for the prevention of cardiovascular diseases were confirmed by Koscielny et al. (1996). Wojcicki et al. (1995) found that an extract of buckwheat plant lowered the risk for arteriosclerosis. Rutin prevents capillary fragility and high blood pressure (Griffith et al., 1944; Schilcher et al., 1990). The antioxidative effect of buckwheat was studied by Watanabe (1998), Park et al. (2000), Holasova et al. (2002) and Morishita et al. (2002). The antioxidative effect of flavonoids was connected with their chemical structure, including the number, location and kind of substitution on the basic flavan molecule (Heim et al., 2002). The diversity of the antioxidative effect was connected to the interaction of genetic and environmental factors in buckwheat (Oomah and Mazza, 1996). Yokozawa et al. (2002) reported that buckwheat polyphenol extract has beneficial effect on patients with renal problems. The effect was probably due to the content of rutin, quercetin, hyperosid and phenolic oligomers of buckwheat. According to findings of La Casa et al. (2000) rutin protected against gastric lesions induced by 50% ethanol.

Nagai et al. (2001) and Gheldof and Engeseth (2002) investigated the antioxidative effects of buckwheat honey and found it had an higher antioxidative ability than honey from other sources. The content of polyphenols is probably connected with the dark colour of buckwheat honey.

Manach et al. (1997) studied the bioavailability of rutin and quercetin. Luthar (1992), Luthar and Kreft (1993) and Hagels (1999a, b) discussed the content of tannin and other polyphenols in buckwheat. It is not yet known if any anti-allergic action of buckwheat grain extract (Kim et al., 2003) is connected with rutin and any related substances or not.

According to Aura et al. (2002) quercetin derivatives are decomposed in the colon to other molecules. Kim et al. (1999) proved that the residue of their decomposition is mainly quercetin.

As reported by Simmering et al. (2002), when food which is consumed is rich in flavonoids, the development of flavonoid degrading microorganisms in the colon is more abundant. Hollman et al. (1995), Gee (2001), Graefe et al. (2001), Wolffram et al. (2002) and Blasiak et al. (2002) studied the absorption of quercetin and its glycosides from the small intestine, of which the sugar part of the complex glycoside molecule seems to be of special importance. After the consumption of buckwheat tea, the quercetin content in blood rises for four hours and then is slowly diminished. Quercetin, along with the vitamin C, may play an important role in the protection of DNA molecules from damage and thus prevent the appearance of mutations and cancer.

Quercitrin is a quercetin 3-glucosid with important antioxidative and antibacterial effects (Wilhelm et al., 2001; Lee et al., 2001c; Arima et al., 2002). Along with rutin, it may protect the cells of the colon (de Medina et al., 2001). An important effect of quercitrin was in diminishing the phototoxicity of hypericin (Wilhelm et al., 2001), a substance of *Hypericum perforatum* L., that is similar to the buckwheat phototoxic substance fagopyrin. Quercitrin was found in Tartary buckwheat by Yasuda et al. (1992), and reported in common buckwheat by Hagels (1999a) and Fabjan (2003).

CONCLUSION

The increase of UV radiation at the Earth's surface, due to the gradual depletion of ozone in the atmosphere (Williamson, 1995), has caused great concern about the consequent effects on ecosystems (Rozema et al., 1997; Häder et al. 1998; Björn, 1999; Gaberščik et al., 2002a; Germ et al., 2002). Mountain habitats are potentially vulnerable to climate changes due to their high elevation. An increase of UV-B radiation (280–320 nm) was reported to range from 6–8% (Caldwell et al., 1980) to 20% (Blumthaler et al., 1993) per 1,000 m of elevation. The production of rutin and other UV-B absorbing compounds in buckwheat was a response to the higher flux of UV-B in the high elevation ecosystems where it originated.

Buckwheat is the only field crop that contains considerable amounts of rutin. The majority of the rutin is synthesized in the herb, especially in the inflorescence. Seeds contain less flavonoids, which are localized mostly in the testa and cotyledons. Green buckwheat tea has become consumed more since it was known for its antioxidative effect, as well as its beneficial effects of rutin on cardiovascular disease, and reducing the risk of arteriosclerosis. Buckwheat is also a nutritional source of quercitrin. There is a need to obtain optimal rutin and quercitrin from buckwheat plants and their products.

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Production and usage of buckwheat grain and flour in Japan

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ABSTRACT

Production and usage of buckwheat grain and flour in Japan were reviewed based on the current and histrorical data on the production and trades of buckwheat grain and flour. Production and utilization (mainly as buckwheat noodles) had the most prosperous era around 1900, but they have declined last 100 years in Japan. Imported grain and groat mainly from China made up the deficit amount from the demand of buckwheat noodle restaurants and manufactures of raw, dried and frozen noodles.

INTRODUCTION

I would like to quote a remark by Mr. Roro Uehara, the author of SOBA Dictionary published in 2002. Uehara (2002) said, "People who love SOBA (buckwheat and/or buckwheat noodles) are happy! When they taste SOBA noodles they can easily image the exquisitely beautiful sceneries of the mountain areas where buckwheat is grown. Also, people who study buckwheat are happy because they have the chance of impressive experience in the way that buckwheat noodle has been developed in its long history."

I have been involved in the buckwheat business at a Japanese trading firm for approximately 20 years, and had the chance to visit buckwheat farms in Japan, China, Canada and the U.S. on many occasions. From this personal experience, I completely agree with Mr. Uehara's remarks on buckwheat. This contemplative view of buckwheat flowering fields make us feel refreshed, whereas buckwheat noodles helps us to maintain our good health.

We are now in the age where advanced analysis technology enables us to identify what food ingredients are beneficial for human health. To me it is amazing, however, that a Japanese medicinal book written over 300 years ago, HONCHO SHOKKKAN (Hitomi, 1697), contains a concise description of the healthful effects of buckwheat. It says, "Intake of buckwheat pacifies one's feelings, relaxes and activates the work of the intestines. It also helps cure swelling, cloudy urine, diarrhea and high blood pressure."

I think that buckwheat is a type of food that is very suitable for the prevention of life style-related adult illnesses. I am proud of my life in the past and at the present, which has been devoted to the buckwheat business and I am very happy to continue my efforts on the enlargement and widespread usage of buckwheat consumption in Japan.

BUCKWHEAT PRODUCTION AND BUCKWHEAT FLOUR CONSUMPTION IN JAPAN

(1) Buckwheat production in Japan

The annual buckwheat production in Japan has been as low as 25,000–30,000 M/T which was harvested from approximately 40,000 ha during the last several years, as indicated in Table 1. This low production is in sharp contrast with the oldest statistical data contained in the archives of the Ministry of Agriculture, Forestry and Fisheries. According to this data Japan had a total buckwheat acreage of approximately 146,000 hectares in 1878 (125 years ago), and 172,000 ha (producing 114,000 M/T) in 1897. The largest total production in Japan, of 139,000 M/T, was achieved in 1907. After 1926 the total buckwheat acreage remained below 100,000 ha and continued to decrease until 1993.

One of the reasons why buckwheat acreage has continued to decrease during this period was the expansion of rice production into lower-temperature areas in the north, such as Hokkaido island and into the more mountainous districts, which was made possible by technical advances in rice production supported by breeding new rice varieties suitable for cool climatic regions. Another reason was the increase in the population of Japan, which led to the replacement of lower yielding buckwheat with higher yielding rice. I believe this was an unavoidable demand of the area and people. According to the statistics in Table 1, the average buckwheat yield was lowest in 1878 at 44 kg/10a and highest in 1960 at 110 kg/10a. It was also quite low during the past 5 years at 64.8 kg. The average buckwheat yield is much lower than ordinary grain crops, however, this drawback is generally well recognized by people because many producers realize that buckwheat is a crop that must be grown on barren land. As a result, Japan is now importing approximately 120,000 M/T of buckwheat grain per year, the details of which I will describe later.

(2) Japanese consumption of buckwheat flour

According to Table 1, Japan had a population of 127.44 million as of October 1, 2002 and in 2002 Japan imported 117,239 M/T of buckwheat grain (whole seed in the hull) which could be milled into approximately 82,070 M/T of flour. In addition to this import of whole grain, buckwheat groats (dehulled seed) were also imported. However, we do not have the exact statistics as they were classified together with dehulled seeds of other grains. However, we can estimate from other information that approximately 13,000 M/T of buckwheat groats were imported in 2002, from which approximately 12,400 M/T of flour could be milled. Therefore, the total quantity of buckwheat flour from both the buckwheat seed and groats imported in 2002 was approximately 94,500 M/T, or 0.74 kg per an individual Japanese consumer.

An average Japanese consumes 32 kg of wheat flour per year, which is approximately 43 times more than an aver-

Table 1. Buckwheat production and utilization in Japan

Year	Acreage (ha)	Acreage in Former Rice Field (ha)	Yield Per 10a	Total Production (M/T)	Import (M/T)	Total supply (M/T)	Conversion to flour (M/T)	Population (million)	Consumption per person (kg) (Seed)
1878	146,000		44	64,300		64,300	45,010	36.17	1.78
1882	157,000		49	77,700		77,700	54,390	37.26	2.08
1887	177,100		80	125,700		125,700	87,990	38.70	3.25
1892	160,500		81	130,100		130,100	91,070	40.50	3.21
1897	172,700		65	114,400		114,400	80,080	42.40	2.70
1902	164,400		65	106,800		106,800	74,760	45.00	2.37
1907	165,300		84	139,300		139,300	97,510	47.42	2.94
1912	145,400		77	112,100		112,100	78,470	50.58	2.21
1916	147,600		89	131,800		131,800	92,260	53.50	2.46
1921	130,400		98	128,400		128,400	89,880	56.67	2.27
1926	107,500		85	91,900		91,900	64,330	60.74	1.51
1930	96,300		109	105,100		105,100	73,570	64.45	1.63
1935	96,200		71	68,300		68,300	47,810	69.25	0.99
1940	83,300		87	72,600		72,600	50,820	71.93	1.01
1945	69,900		48	33,800		33,800	23,660	72.15	0.47
1950	68,000		76	52,000		52,000	36,400	84.11	0.62
1952	57,000		92	52,300	150	52,450	36,715	85.81	0.61
1955	48,000		82	39,300	4,041	43,341	30,340	90.08	0.48
1960	47,300		110	52,500	0	52,500	36,750	94.30	0.56
1963	37,500		108	40,500	13,760	54,260	37,982	96.15	0.56
1965	31,300		96	30,100	13,223	43,323	30,320	99.21	0.44
1970	18,500		93	17,200	44,623	61,823	43,270	104.66	0.59
1975	18,300	5,300	101	18,400	47,418	65,818	46,070	111.94	0.59
1980	24,200	15,600	67	16,100	66,698	82,798	57,950	117.06	0.71
1982	23,700	15,900	80	19,100	87,178	106,278	74,400	118.73	0.89
1985	18,700	11,100	93	17,300	75,114	92,414	64,690	121.05	0.76
1989	25,900	17,500	79	20,500	96,461	116,961	81,870	123.20	0.95
1993	22,600	12,800	69	15,600	99,755	115,355	80,750	124.94	0.92
1998	34,400	23,300	52	17,900	99,359	117,259	82,080	126.49	0.93
1999	37,100	23,600	65	24,000	103,290	127,290	89,100	126.69	1.00
2000	37,400	25,400	78	29,200	97,050	126,250	88,370	126.93	0.99
2001	41,800	29,600	65	27,300	92,722	120,022	84,010	127.29	0.94
2002	41,400		64	26,600	90,639	117,239	82,070	127.44	0.92

age Japanese consumes of buckwheat flour. More than one hundred years ago, an average Japanese consumed a little over 2 kg of buckwheat flour per year (Table 1), or nearly 3 times more than now. We should remember two old sayings inherited from our forefathers; "People who love buckwheat live long" and "People who love buckwheat are healthy." It is my belief that we should never forget what these old sayings are telling us about the benefits of buckwheat. It would be very beneficial if we could compare the life expectancies of Japanese people one hundred years ago and now and correlate it with buckwheat consumption. Of course, it would be difficult to prove the exact correlation between the quantity of buckwheat flour consumption and life expectancy even now, but such efforts may greatly help the Japanese perceive the true benefits of buckwheat.

UTILIZATION OF BUCKWHEAT GRAIN IN JAPAN

(1) Domestic buckwheat

Buckwheat acreage in Japan was over 160,000 ha about 100 years ago. However, it continuously declined to less than 20,000 ha about 30 years ago, and then it increased to the present level of 40,000 ha. This limited recovery in buckwheat acreage was not due to the increased preference of buckwheat production by farmers, but was due to the subsidies granted by the Japanese government to farmers who grew buckwheat and other crops, to decrease the amount of excessively produced rice. Buckwheat production has ranged between 24,000 t and 30,000 t during the past 4 years, but it is estimated that as much as 40% of it is milled and consumed in and around each production area. This tendency is due to the nationwide campaign of utilization of buckwheat and other crops for local economic promotion and is partly due to the choice of the local people to use more local products that they are familiar with to ensure safety of the food they consume daily. The remaining 60%, or more exactly 53% after deducting 7% that is used for planting purpose, is collected from the producers, packed in bags and stored by local agricultural cooperatives or local grain dealers after adjustment of the moisture content and the removal of small stones and other unfavorable materials plus any immature seed. The adjustment of the moisture content is a crucial process in Japan where it rains a lot in the fall when buckwheat is being harvested, and it is conducted either by the farmers utilizing drying machines usually used for drying rice in the husk or is dried by agricultural cooperatives or local grain dealers using large-scale drying machines. We are unable to determine at the present time which drying method is better. The grain, which is packed in bags, is then sold by the local cooperatives and grain dealers to buckwheat millers in the consuming areas through wholesale dealers. However, recently an increasing number of buckwheat noodle restaurants have started to buy buckwheat grain directly from the producing areas and mill it in-house by themselves.

(2) Imported buckwheat seed

Buckwheat grain was first imported to Japan 51 years ago in 1952. In that year a total of 150 M/T was imported from South Africa. The importation of buckwheat grain has increased, except in some years, in proportion to the decline of domestic buckwheat production until 2000, when it started to decrease. As already mentioned before, buckwheat grain does not include groats under the Japanese system of statistics. This decrease in the importation of buckwheat grain is due to the increase of importation of buckwheat groats from China. Consequently, the Japanese importation of buckwheat "grain" in 2002 dropped to 90,639 M/T with a breakdown of 79,999 M/T (88.2%) from China, 5,632 M/T (6.2%) from the U.S., 3,625 M/T (4%) from Canada and 1,383 M/T (1.5%) from other countries. However, from a more practical perspective, we can say that the total Japanese importation of buckwheat in 2002 was 107,939 M/T consisting of 90,639 M/T of "grain" plus an additional 17,300 M/T of "grain" as calculated from the estimated 13,000 M/T of "groats" that were imported. Of this calculated total import of "grain" of 107,939 M/T, we can say that 97,299 M/T (90.1%) came from China. This situation shows a sharp contrast with that of 1982, when the total buckwheat grain imported was 66,698 M/T, with a breakdown of 32,863 M/T (49.3%) from Canada, 17,525 M/T (26.3%) from China, 7,645 M/T (11.5%) from the U.S. and 8,665 M/T (12.9%) from other countries. What an enormous advance China made in buckwheat exports to Japan during the 20 years between 1982 and 2002!

The buckwheat grain shipped by exporters from the countries of origin are imported by Japanese trading firms. It is then distributed to Japanese millers through grain dealers in almost the same way as is the domestic buckwheat grain.

(3) Some characteristics of buckwheat seed transactions in Japan

Because of the need to secure sufficient buckwheat grain over different time periods as raw material for the buckwheat dealers, they have to make purchases of both overseas and domestic buckwheat seed well in advance of their needs. It is currently common practice to make such purchases each year during June/July after seeding is over. However, there were many cases, up to about 10 years ago, in which dealers used to start making purchases even a few months prior to seeding. No public hedging systems were or are available in Japan for buckwheat, hence serious situations could or did occur in years of poor harvest, including non-delivery of purchase orders. This may also be one of the reasons why more substantial development of the Japanese buckwheat industry has been limited.

CIRCULATION AND USE OF BUCKWHEAT FLOUR IN JAPAN

(1) Noodles

The fact that over 90% of the flour manufactured by millers is consumed as noodles characterizes the major usage of buckwheat flour in Japan. Most of the buckwheat flour is sold by the millers directly to buckwheat noodle restaurants and to manufacturers of raw, dried or frozen noodles. A small part of the buckwheat flour produced is also marketed by some foodstuff wholesalers. The types of buckwheat noodles that people consume include noodles served at buckwheat noodle restaurants, and the boiled, raw, dried, frozen and precooked "instant noodles" that they buy at other places, such as convenience stores and supermarkets.

According to data from the Processed Food Division of the Ministry of Agriculture, Forestry and Fisheries a total of 47,453 M/T of buckwheat flour was used in 2002 to manufacture raw, boiled, dried and frozen noodles. The total buckwheat flour that was available in 2002 was 94,500 M/T as was calculated before. Deducting the 47,453 M/T used for noodle production as well as approximately 7,000 M/T used for purposes other than noodles, we find that about 40,000 M/T was used by noodle restaurants in 2002. Dividing 40,000 M/T by 30,000 (the number of noodle restaurants in Japan) we find that the average noodle restaurant uses only 1.333 M/T a year or 111.1 kg per month. Assuming that a single serving at a noodle restaurant normally contains 70 g of buckwheat flour and 30 g of wheat flour we may estimate that the 111.1 kg flour which is used monthly by the average noodle restaurant is enough to serve only 1,587 customers or 61 customers per day that they are open. This indicates that a total of 571 million servings of buckwheat noodles are annually provided at noodle restaurants in Japan, which is equal to just 4.5 servings for the average Japanese. The figures seems unbelievably small to me. It is my firm belief that Japanese people should consume at least as much buckwheat as in the Meiji Era (1868–1911) to maintain our good health and I will continue to devote my efforts to attaining this target.

(4) Use of buckwheat flour for non-noodle purposes

Other current uses of buckwheat, other than for noodle production, is for the production of alcoholic drinks and buckwheat tea. Very little buckwheat flour is currently being used for production of cookies and Japanese sweets. The quantity used in this field may be roughly 7,000 M/T, however, an exact estimate is exceedingly difficult to obtain.

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Morphological variation and differentiation between diploid and tetraploid cytotypes of *Fagopyrum cymosum*

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Key words: *Fagopyrum cymosum*, morphology, perennial buckwheat, polyploidy, Principal Component Analysis (PCA)

ABSTRACT

A comprehensive morphological comparison between diploid and tetraploid *F. cymosum* was conducted. Among the 17 characters investigated only the seed weight critically distinguished between the two ploidies. Variances in all the characters investigated among the populations, except for the hypocotyl elongation, were higher in the diploid than in the tetraploid. The data was also analyzed by the Principal Component Analysis (PCA). Diploid populations varied more widely than did the tetraploid populations on the first principal component (PC1). Tetraploid populations were divided into two geographically diverged groups along the PC2. This result coincides with previous studies done by allozymes and nucleotide sequences of cpDNA, which suggested the hypothesis of muliple origins of tetraploid *F. cymosum*.

INTRODUCTION

Fagopyrum cymosum Meisn., commonly known as perennial buckwheat, is a herbaceous, perennial, insect-pollinated heterostylous species. Its habitat is forest margins, around roadsides, and near housing areas in the countryside (Fig. 1). Usually the species forms small clumps, either as a small population consisting of a few tens to one hundred individuals or as a larger population. Both diploid (2n=2x=16, Darlington and Wylie, 1955) and tetraploid (2n=4x=32, Sharma and Chatterji, 1960) cytotypes are known. Diploid *F. cymosum* is mainly distributed in the Sichuan and Yunnan provinces and in eastern Tibet in China, whereas tetraploid populations are more widely distributed in southwestern China, Thailand, the Himalayan hills and Tibet (Ohnishi, 1998; Tsuji et al., 1999). Diploid and tetraploid types of *F. cymosum* are morphologically so similar to each other that they are included in a single species, *F. cymosum*. Some morphological differences were found between two ploidy types in *F. cymosum*; e. g. seed size (larger in diploid, see Fig. 2) and rhizome forms (woody bulbs in diploid) (Campbell, 1976; Yasui, 1991). However, no morphological diagnostic characters that can discriminate between the diploid and tetraploid cytotypes in *F. cymosum* have not been established to date on the basis of an extensive morphological survey on samples covering entire geographic distribution areas.

In order to clarify the morphological differentiation among local populations of *F. cymosum*, Yasui (1991) investigated the leaf form of four populations, three diploid populations from Dujiangyang (Sichuan province), Lijiang and Kunming (Yunnan province), and a tetraploid population from Dali (Yunnan province). He noticed that the differentiation of this character among local popula-

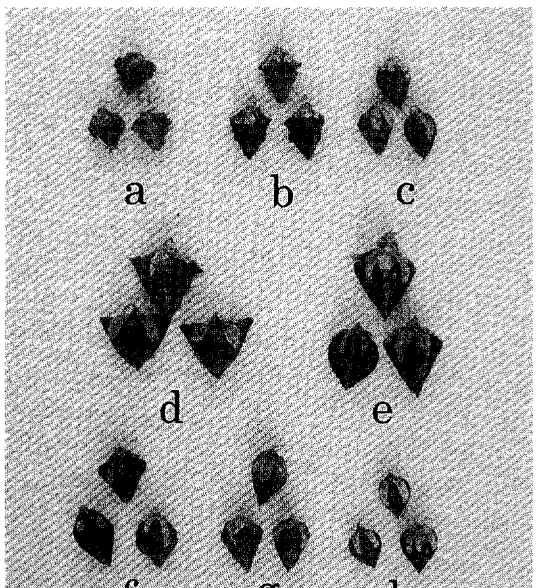

Fig. 2. Seed morphology of *F. tataricum* and *F. cymosum*.
a: *F. tataricum* ssp. potanini from Leguhu in Yunnan province
b: diploid *F. cymosum* from Tongmai in eastern Tibet (similar to *F. tataricum*)
c: diploid *F. cymosum* from Tongmai in eastern Tibet (similar to common *F. cymosum*)
d: diploid *F. cymosum* from Dujiangyang in Sichuan province
e: diploid *F. cymosum* from Xiaguan in Yunnan province
f: tetraploid *F. cymosum* from Zhangmu in Tibet
g: tetraploid *F. cymosum* from Yuehua in Sichuan province
h: tetraploid *F. cymosum* Dali in Yunnan province

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tions was remarkable, and that the diploid population from Dujiangyang was quite different from the other diploid populations. However, he investigated a limited number of populations, which did not cover the distribution areas of the species. Recently, allozymes (Yamane and Ohnishi, 2001) and nucleotide sequences of cpDNA (Yamane et al., 2003) were utilized to evaluate the genetic variation and to clarify the evolutionary processes of tetraploidizaton in *F. cymosum*. The results of these studies suggested the following; (1) teraploid *F. cymosum* maintains a larger amount of allozyme and cpDNA variation than the diploid does, (2) *F. cymosum* was highly differentiated among populations, and (3) tetraploid *F. cymosum* originated allopatrically. For a comprehensive understanding of the evolutionary history of the species, it was necessary to assess the morphological variation and differentiation among local populations.

Seventeen morphological characters were investigated in populations covering the wide geographical distribution areas of *F. cymosum* to reveal (1) geographic patterns of morphological variation among local populations; (2) morphological differentiations between diploid and tetraploid populations; (3) possible diagnostic character(s) between diploid and tetraploid populations.

MATERIALS AND METHODS

Plant materials

Twenty-one populations, 11 diploid and 10 tetraploid, of *F. cymosum* were carefully selected from the collection of the Laboratory of Crop Evolution, Graduate School of Agriculture, Kyoto University so as to cover the entire range of the geographic distribution of the species (see Table 1 and Fig. 3). The original seed samples (100–1000 seeds) were collected from natural populations by Ohnishi, Yasui and Tsuji during their expeditions in 1983–1998 (Ohnishi, 1998; Ohnishi and Yasui, 1998; Tsuji, et al., 1999). The plant materials were grown for each population in a field of the Laboratory of Crop Evolution, Graduate School of Agriculture, Kyoto University at Muko City, Kyoto Prefecture in Japan in June-September in 1998. On average, twenty individuals per population were investigated for this study.

Morphological characters

Seventeen characteristics for leaf color, pubescence, anthocyanin pigmentation, leaf form, plant type, hypocotyl elongation, and seed weight were scored on each individual (Table 2). Hypocotyl elongation (elongated or not) was checked at the seedling stage (Fig. 4). For the

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

Accesstion	Abbreviation	Ploidy	Village town or city	Sites, Province of Distinct	Country	Latitude of collection site (°N)
C9141	C1	2X	Dujiangyan	Sichuan	China	31
C9438	C2	2X	Luding	Sichuan	China	29.8
YC9436	C3	2X	Kangding	Sichuan	China	30
C9437	C4	2X	Muli	Sichuan	China	27.8
C9434	C5	2X	Qiaotou	Yunnan	China	27.2
C9656	C6	2X	Yongsheng	Yunnan	China	26.7
C9747	C7	2X	Heqing	Yunnan	China	26.5
C9435	C8	2X	Wenbixian	Yunnan	China	26.8
C8926	C9	2X	Songming	Yunnan	China	25.3
C8924	C10	2X	Xiaguan	Yunnan	China	25.5
YC 9806	C14	2X	Tongmai	Tibet	China	30
C8822	C11	4X	Dali	Yunnan	China	25.5
C9749	C12	4X	Baoshan	Yunnan	China	25.1
C8927	C13	4X	Anshun	Guizhou	China	26.2
T8502	T1	4X	Doistephe	Chieng Mai	Thailand	20
C9248	C15	4X	Zhangmu	Tibet	China	29
B9103	B1	4X	Thimphu	Thimphu	Bhutan	27.5
N8641	N1	4X	Chouri Kharka	Salukhumbu	Nepal	28.5
N8367	N2	4X	Shikha	Myagdi	Nepal	29.5
I8644	I1	4X	Gwaldon	Utter Pradesh	India	30.5
I8642	I2	4X	Kathi	Utter Pradesh	India	30.5

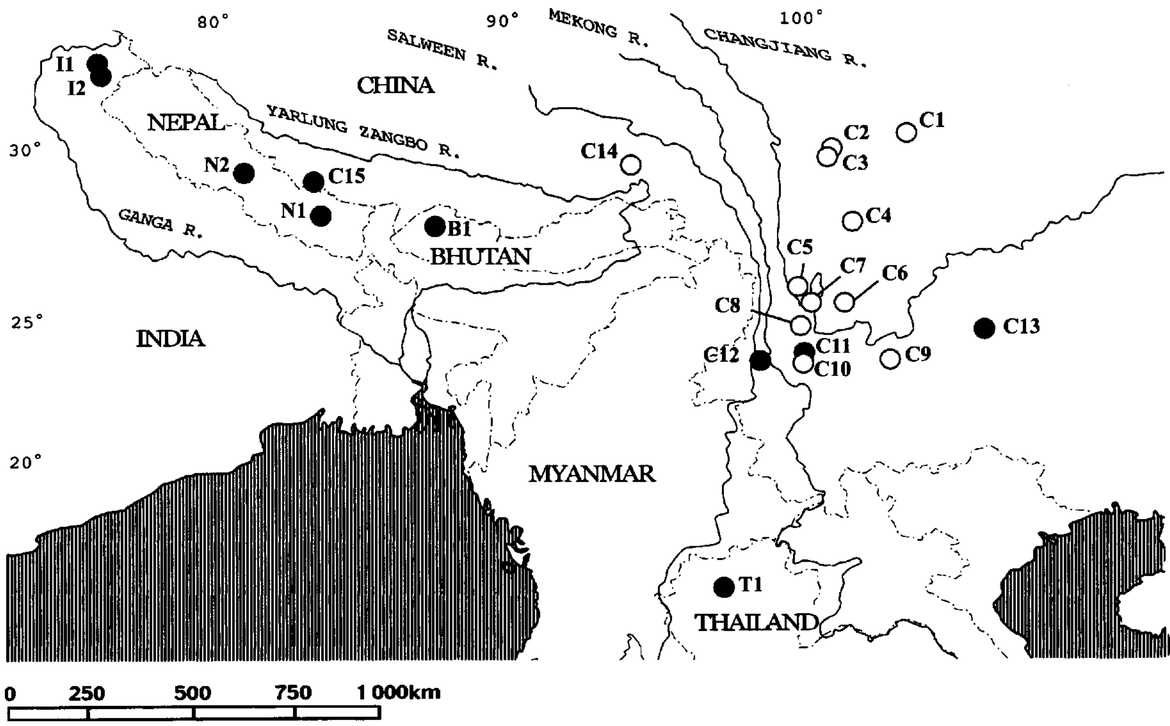

Fig. 3. The locations of sampling sites for plants used in the present study. Open and closed circles indicate diploid and tetraploid populations, respectively. See Table 1 for abbreviation codes.

Table 2. Characters used in the morphological analysis of *F. cymosum*

No.	Morphological character	Unit or Category
1	Hypocotyl elongation	non elongation=0, elongated=1
2	Leaf color (hue)	0–10*1 (reference form standard leaf color charts)
3	Leaf color (value)	0–10*1 (reference form standard leaf color charts)
4	Leaf color (chroma)	0–10*1 (reference form standard leaf color charts)
5	Leaf pubescence (surface side)	none=0, most heavily=3
6	Leaf pubescence (reverse side)	none=0, most heavily=3
7	Petiole pubescence	none=0, most heavily=3
8	Vein pubescence (surface side)	none=0, most heavily=3
9	Vein pubescence (reverse side)	none=0, most heavily=3
10	Anthocyanin pigmentation of petiole (surface side)	no pigmentation=0, deep pigmentation=4
11	Anthocyanin pigmentation of petiole (reverse side)	no pigmentation=0, deep pigmentation=4
12	Anthocyanin pigmentation of vein (surface side)	no pigmentation=0, slightly pigmentation=1, deep pigmentation=2
13	Anthocyanin pigmentation of vein (reverse side)	no pigmentation=0, slightly pigmentation=1, deep pigmentation=2
14	Anthocyanin pigmentation at root of vein	no pigmentatione=0, deep pigmentation=3
15	Plant type	0–6*2
16	Leaf form	leaf width/length of auricle*3
17	Seed weight	g/seed

*1: referred from the Standard Leaf Charts. *2: see Fig. 6 and 7. *3: see Fig. 5.

leaf characters, the fifth to seventh leaves on the main stem were measured for each individual. Pubescence and anthocyanin pigmentation were checked on the petiole and at the leaf veins. The leaf form was characterized as a ratio of auricle depth to leaf width (see Fig. 5). The leaf color was determined referring by to the Standard Leaf Color Charts (Japan Color Research Institute, Japan). The plants were classified into 7 types based on different types of shoots they possessed (e. g. straight or non-straight, erect or creeping, the degree of ramifications, see Figs. 6 and 7). Seed weight was measured on twenty original seeds that had been collected from the natural populations.

Data analyses

A morphological comparison between diploid and tetraploid *F. cymosum* was made using one-way analysis of variance (ANOVA). Principal component analysis (PCA) was used to clarify the factors or components responsible for difference. For this analysis we used the average values of populations for each characters. All analyses were performed using the Microsoft Excel Statistical Analysis System Program (2000) for Windows.

RESULTS

Morphological variations

The means of the measurements of 17 characters for each population are shown in Table 3. The mean and variance of the17 characters were compared between diploid and tetraploid types of *F. cymosum* (Table 4). Of the 17 characters measured, the following seven characters showed a significant difference between the diploid and tetraploid cytotypes: leaf color, leaf pubescence (surface and reverse side), pubescence on the vein (surface side), anthocyanin pigmentation of petiole (surface and reverse side), and seed weight (Table 4). The hypocotyl elongation was observed only on several individuals of a few tetraploid populations. The percentage of elongated individuals in a population was 100% in T1, 70% in C11, 50% in C12, and 20% in C13, but no individual from any tetraploid populations from the Tibet-Himalayan regions showed this character. The seed weight (g) ranged from 0.030–0.048 in the diploid populations and 0.019–0.026 in tetraploid populations. This character critically distinguished the two ploidies without any overlaps of individual values. Variances of all the characters investigated, except for the hypocotyl elongation, (the variance of this character was zero in diploid), were higher in the diploid populations than in the tetraploid populations (Table 4).

Principal Component Analysis

In PCA, the first three components took 51.5%, 12.4% and 9.1% of the total variance, respectively (Table 5). Leaf pubescence (on the surface and on the reverse side), pubescence on the vein (surface side and reverse side), and pubescence on the petiole made large contributions to the first component (PC1) (Table 5). The second principal component (PC2) was mainly contributed from hypocotyl elongation, and leaf color (chroma and value)

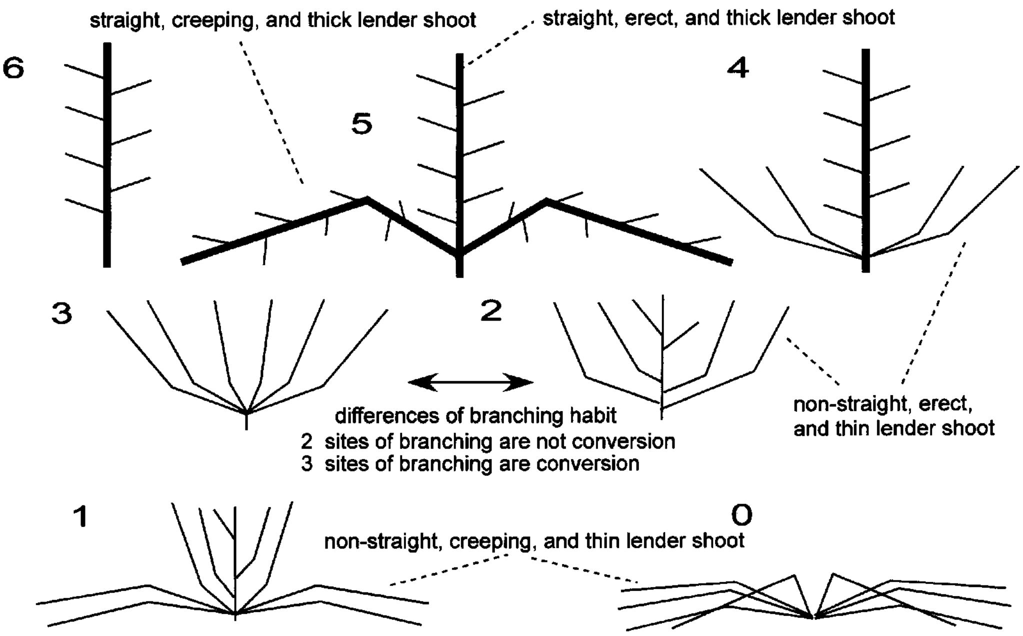

Fig. 6. Seven plant types in *F. cymosum*. The features of the shoot were distinguished by using the following three criteria; straight or non-straight, erect or creeping, thick or thin. Types of No. 6, 5 and 4 have thick slender shoot (bold line). Types of No. 5, 1 and 0 have creeping shoot. All shoots were creeping in type No. 0.

Fig. 1

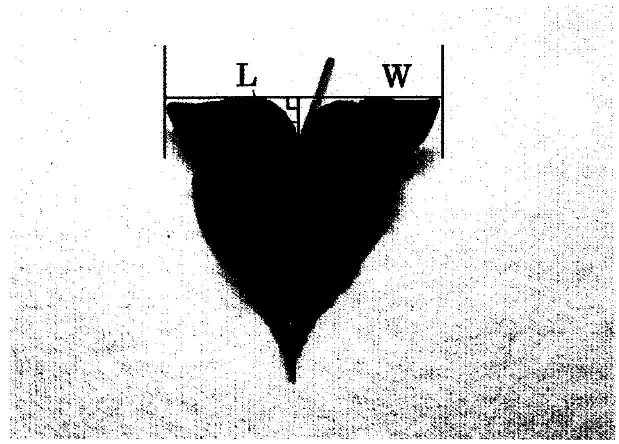

Fig. 4

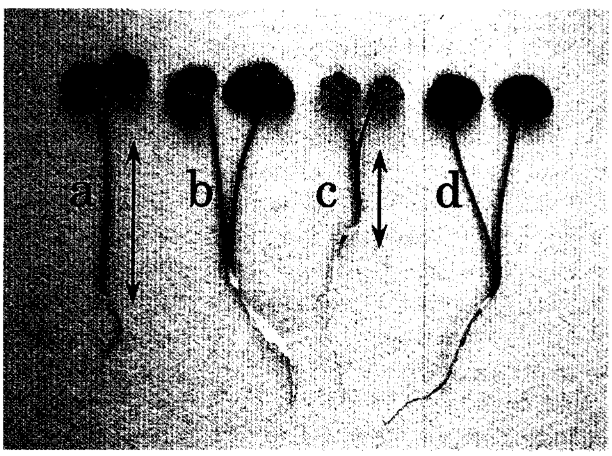

Fig. 5

Fig. 1. A natural population of tetraploid *Fagopyrum cymosum* at Dali city in Yunnan province, China.

Fig. 4. Hypocotyl elongation of *F. cymosum* and *F. tataricum*.
a: *F. tataricum* ssp. *tataricum* from Qidian in Yunnan province
b: diploid *F. cymosum* from Xiaguan in Yunnan province
c: tetraploid *F. cymosum* from Dali in Yunnan province
d: tetraploid *F. cymosum* from Zhangmu in Tibet
Elongation of hypocotyl was observed in a and c, which are indicated by an arrow.

Fig. 5. Measurement of leaf characters. L: auricle depth, W: leafwidth. The ratio of W/L was used as a leaf character (after Yasui, 1991).

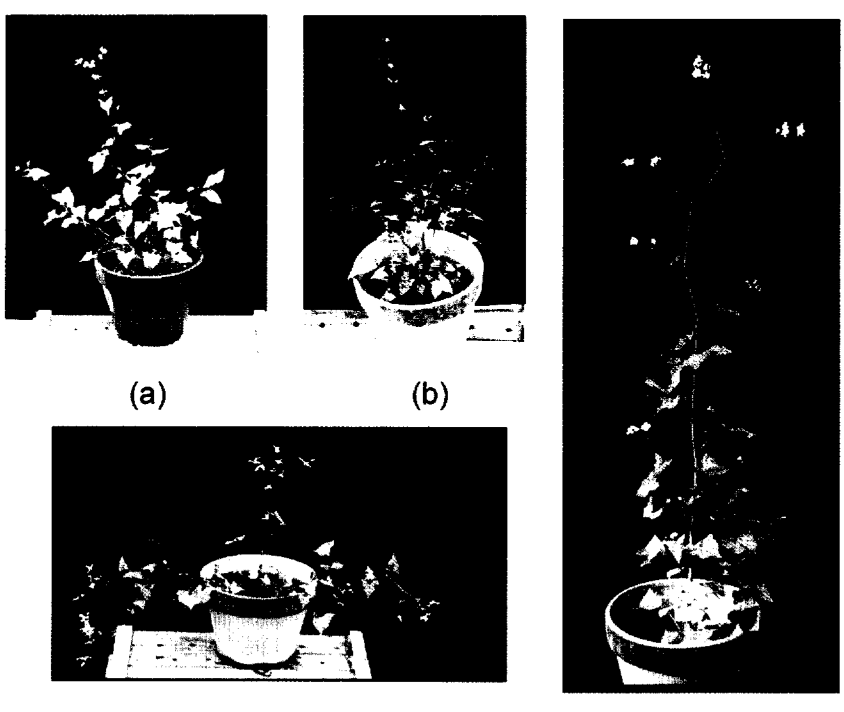

Fig. 7. Plant types of *F. cymosum*. (a) Tetraploid from Kathi in India. (b) Diploid from Tongmai in eastern Tibet. (c) Diploid from Muli in the Sichuan province of China. (d) Tetraploid from Anshun in Guizhou province of China.

Table 3. Means of 17 morphological characters for all populations in *F. cymosum*.

	Character No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Diploid populations	C1	0	7.5000	3.8810	4.6905	0	0	0.0476	0.9048	0.6667	1.7619	0.4286	0.7143	0.1429	1.2857	4.0000	8.3175	0.0332
	C2	0	6.0185	4.3704	5.5556	2.1111	1.5556	2.3333	1.5926	2.3704	2.5926	0.7778	1.6296	1.9259	2.2593	5.8000	8.1144	0.0439
	C3	0	7.0833	4.2778	4.9167	1.9444	0.9444	1.6111	1.3333	2.0000	1.0000	0.1667	0.8333	0.9444	1.9444	5.5000	8.7817	0.0453
	C4	0	6.7593	4.4074	5.3519	1.8148	1.7037	1.6296	1.6667	2.2222	1.1852	0.7037	1.1852	0.8519	1.2963	5.0000	7.2752	0.0410
	C5	0	6.3889	4.2593	5.4630	2.0741	1.0741	2.0741	1.1481	1.9259	1.3704	0.3704	1.0370	0.8148	1.6296	3.0000	6.6642	0.0373
	C6	0	7.5000	4.2143	4.9071	0.8095	0.3333	0.6190	1.0000	1.3333	1.7619	0.5238	0.9524	0.3333	1.2381	4.4000	4.4426	0.0432
	C7	0	6.7857	4.4524	5.0000	0.9524	0.0952	1.0952	0.8571	1.1905	1.6190	0.5238	1.0952	0.8571	1.2381	2.0000	4.3983	0.0526
	C8	0	6.3542	4.3542	5.3021	1.6250	1.3542	1.8958	1.4583	2.2292	1.1250	0.3750	1.2917	0.7500	1.0833	3.0000	3.2313	0.0502
	C9	0	7.0536	4.2679	5.1518	1.0714	0.5179	0.8750	1.2679	1.6429	0.6429	0.3214	1.0179	0.2143	1.1964	0	5.0608	0.0323
	C10	0	7.3958	4.0208	5.0208	0.7083	0.0833	0.6250	0.9167	1.0000	1.3750	0.2500	0.6667	0.2917	1.3750	0.7500	4.5714	0.0480
	C14	0	7.5000	3.6667	4.5000	1.2857	0.4286	1.0000	0.7143	1.5714	0.8571	0	0.8571	1.0000	1.5714	1.2143	2.6345	0.0020
Tetaraploid populations	C11	0.7000	7.5000	4.1406	5.0156	1.0000	0	0.8125	1.0000	1.0625	1.2500	0.1563	0.9375	0.1563	1.0000	0.7059	4.7105	0.0199
	C12	0.5000	7.1296	4.2222	5.2407	0.9259	0	0.8519	1.0000	1.1852	1.2963	0.2963	0.9630	0.3333	1.1852	1.1670	3.7258	0.0180
	C13	0.2000	7.0000	4.4000	5.4667	0.3333	0	0.6000	0.8000	0.9333	1.3333	0.1333	0.8667	0.4000	1.5333	0.5882	4.0152	0.0256
	C15	0	7.4359	4.0641	5.0513	0.9231	0.0769	1.4359	0.9231	1.6923	0.7692	0.0256	0.7692	0.5128	1.4103	3.0000	5.1316	0.0178
	T1	1.0000	7.1875	4.2083	5.2708	0.7500	0.1250	0.7500	0.8333	1.3750	0.7083	0.1250	0.6667	0.4167	0.6250	1.0000	6.4357	0.0159
	B1	0	7.5000	3.8810	5.0238	1.0000	0.3810	1.2381	1.0000	1.5238	1.1905	0.1905	1.1429	0.8571	1.1905	2.0000	4.6849	0.0241
	N1	0	7.4405	4.0119	5.0357	0.8333	0.0476	1.2381	0.8571	1.2857	1.0000	0.2143	0.8095	0.4048	1.3810	3.0000	6.0506	0.0292
	N2	0	7.5000	4.0333	4.9667	0.5333	0	0.9333	0.7333	1.2667	0.1333	0.0000	0.1333	0.4000	0.9333	2.3333	4.3255	0.0295
	I1	0	7.5000	3.9394	4.8030	1.0000	0.1212	0.8788	0.9697	1.2121	1.0606	0.3636	0.8788	0.6970	1.7273	3.0000	3.7010	0.0242
	I2	0	7.4242	4.0000	5.1364	0.8182	0	0.8182	0.7879	1.0909	0.6061	0.1818	0.8788	0.5758	1.3030	3.0000	4.7418	0.0231

Population abbreviation and Characters No. was shown in Table 1 and 2, respectively.

Table 4. Mean and variance of morphological characters in *F. cymosum*. F-values for univariate differences between diploid and tetraploid cytotypes. Statistical differences among two cytotypes determined by ANOVA.

	Character No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
2X	Mean value	0	6.940	4.197	5.078	1.309	0.735	1.255	1.169	1.650	1.390	0.404	1.025	0.739	1.465	3.151	5.772	0.039
(N=11)	Minimum value	0	6.589	4.034	4.860	0.858	0.320	0.780	0.955	1.277	1.029	0.252	0.840	0.400	1.224	1.824	4.331	0.030
	Maximum value	0	7.291	4.361	5.296	1.759	1.151	1.730	1.383	2.024	1.751	0.556	1.211	1.078	1.706	4.479	7.213	0.048
	Variance	0	0.249	0.054	0.096	0.409	0.348	0.455	0.092	0.281	0.262	0.046	0.069	0.232	0.117	3.550	4.180	0.000
4X	Mean value	0.240	7.362	4.090	5.101	0.812	0.075	0.956	0.890	1.263	0.935	0.169	0.805	0.475	1.229	1.979	4.752	0.023
(N=10)	Minimum value	-0.022	7.230	3.980	4.968	0.653	-0.010	0.769	0.818	1.102	0.663	0.090	0.613	0.335	1.001	1.246	4.097	0.019
	Maximum value	0.502	7.494	4.201	5.234	0.970	0.160	1.143	0.962	1.423	1.207	0.247	0.996	0.616	1.456	2.713	5.408	0.026
	Variance	0.120	0.031	0.021	0.031	0.044	0.013	0.062	0.009	0.045	0.130	0.011	0.064	0.035	0.091	0.947	0.755	0.000
	F value	—	5.822	1.422	0.038	4.979	10.965	1.587	6.991	4.221	4.929	8.868	0.221	2.385	2.528	1.172	1.933	12.393
	P value	—	0.026*	0.248	0.847	0.038*	0.004**	0.223	0.016*	0.054	0.039*	0.008**	0.079	0.139	0.128	0.110	0.180	0.002**

N=number of populations. *P<0.05, **P<0.01

(Table 5). Fig. 8 shows the distribution of 21 populations on the plain of the first two components. Diploid populations varied more widely than did the tetraploid populations on the PC1 axis. Moreover, tetraploid populations were divided into two geographically diverged groups along the PC2; the Yunnan-Sichuan region and the Tibet-Himalayan region.

DISCUSSION

Morphological variations and differentiations among local populations

In the present study, the leaf form was measured using the same method of Yasui (1991) and the present results were perfectly consistent with his results, i. e. the mean

Table 5. Cumulative variances of first and second principle components.

Morphological character	PC1	PC2
Leaf pubescence (reverse side)	0.918	–0.012
Vein pubescence (sursurface side)	0.886	0.112
Leaf pubescence (surface side)	0.849	–0.066
Petiole pubescence	0.845	–0.033
Vein pubescence (reverse side)	0.843	–0.025
Antcyanin pigmentation of vein (surface side)	0.787	0.104
Antcyanin pigmentation of vein (reverse side)	0.773	–0.312
Antcyanin pigmentation of petiole (reverse side)	0.737	0.059
Plant type	0.656	–0.477
Leaf color (value)	0.605	0.638
Seed weight	0.599	0.063
Antcyanin pigmentation at root of vein	0.585	–0.568
Leaf color (chroma)	0.571	0.656
Antcyanin pigmentation of petiole (surface side)	0.562	–0.055
Leaf form	0.480	–0.138
Hypocotyl elongation	–0.311	0.674
Leaf color (hue)	–0.872	–0.327
Cumulative variance %	51.52%	12.39%

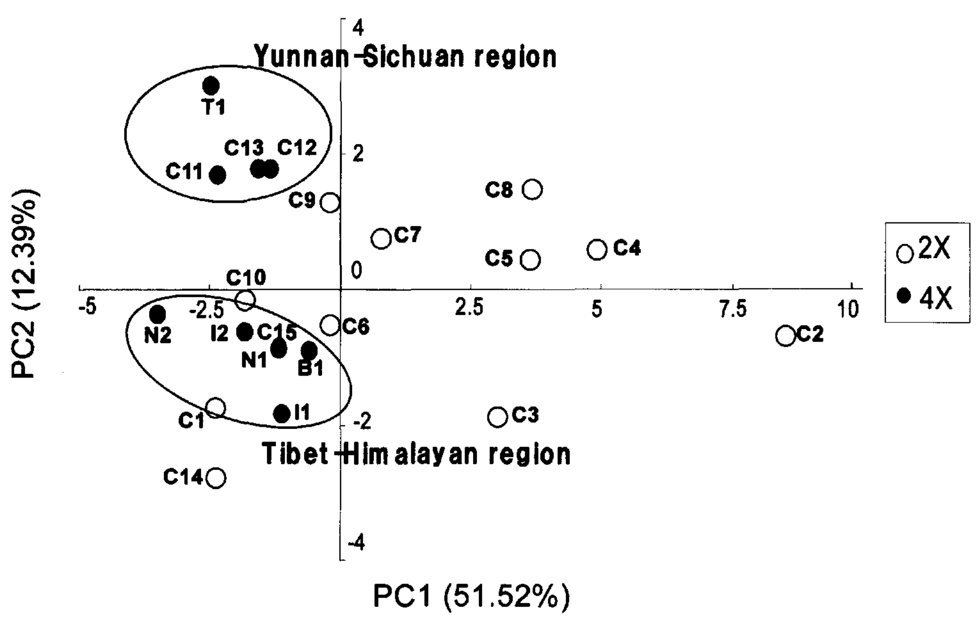

Fig. 8. Scatter plot of PCA of the populations on the first two principal axes. Open circle=diploid, closed circle=tetraploid. For abbreviations for populations, see Table 1.

values of the populations were 8.37 (C1, Sichuan province), 4.57 (C10, Yunnan province), and 4.71 (C11, Yunnan province). By investigating a larger number of populations in this study than was done in Yasui's study (1991), it clarified that leaf form showed a geographically continuous variation among the local populations (see Table 3). This geographically continuous variation among local populations was also found in other characters. For example, the mean value of pubescence on the leaf gradually increased in northern populations. This tendency was found in those characters that contributed highly to the PC1 axis (see Table 3 and 5). Moreover, the PC1 axis and the geographic position (mainly their latitude, see Table 1) of the diploid populations were correlated; with the correlation coefficient being 0.281 (not significant). However, if the Dujiangyang population is excluded, the correlation coefficient becomes significant at 0.778 ($p<0.005$). The reason for the different results may be that the value of pubescence, which highly contributed to the PC1, was exceptionally low in the Dujiangyang population in spite of its high latitude (see Table 3). This suggested that morphological characters (e.g. pubescence and pigmentation by anthocyanin; see Table 5) that contributed highly to the PC1 axis might have been affected by the latitude of the populations. Two alternative explanations are possible; one is natural selection by some factors related to latitude, and the other is possible migration of populations from south to north (or vice versa). Although the characters of pubescence and pigmentation by anthocyanin may be affected by environmental changes, there was no means to confirm this hypothesis in this study.

Morphological differentiation and diagnostic character between diploid and tetraploid *F. cymosum*

Tetraploid populations, which are more widely distributed in various habitats, might differ in their degree of phenotypic plasticity for crucial environmental fluctuation. However variances of most of their morphological characters were found to be smaller in tetraploid populations than in diploid ones. This is probably due to the well-known "buffering effect by polyploidization" (see Stebbins, 1956), that is, each gene affecting a quantitative character makes a smaller contribution to the variation at the tetraploid level than at the diploid level. On the other hand, the most widespread effect of polyploidy is an increase in cell size (gigas-effect; see Lewis, 1980). The number of cell divisions during growth and development is reduced in polyploids, and thus polyploids grow and develop slower than the parental diploids. This gigas effect is often found in flowers and seeds (Lewis, 1980). Nevertheless, the seed weight of tetraploid populations was unexpectedly significantly lower than that of the diploid ones in *F. cymosum*. This is a well-known controversial phenomenon in buckwheat. At the present time we have no idea of how to explain this phenomenon.

Out of the 17 morphological characters investigated, only seed weight could be used as a diagnostic character to discriminate between diploid plants and tetraploid plants in *F. cymosum*. This character was used as a key character to distinct diploid and tetraploid *F. cymosum* by Yasui (1991). Other characters could not clearly distinguish between diploid and tetraploid types. As mentioned in the section of INTRODUCTION, rhizome forms (woody bulbs in diploid) (Campbell, 1976; Yasui, 1991) are well known as a discriminating character between two ploidy types in *F. cymosum*, however, we did not investigate this character in this study, because it was difficult to determine this character on first year plants of perennial buckwheat. In the two dimentional (PC1 and 2) plot of populations, the tetraploid populations could not be separated from the diploid populations (Fig. 8), indicating that morphological discrimination between diploid and tetraploid cytotypes is difficult.

Two distinct groups of tetraploids populations

Two groups recognized in the PC2 axis coincide with the geographical groups of tetraploid populations separated by the Hengduanshan mountains as suggested by allozyme analysis (Yamane and Ohnishi, 2001) and the analysis of cpDNA (Yamane et al., 2003); one consists of the populations from Tibet and the Himalayan hills, Nepal, Bhutan, and India (Tibet-Himalayan group), and the other consists of the populations from Yunnan, Sichuan, and Guizhou provinces of China, and Thailand (Yunnan-Sichuan group). Two alternative explanations are possible for this result; one is of an allopatric independent origin of the two tetraploid populations, and the other is that morphological differentiation occurred by natural selection due to different environmental conditions between the two geographically isolated groups of populations. The previous molecular studies (Yasui and Ohnishi, 1998a, b; Yamane and Ohnishi, 2001; Yamane et al., 2003) clearly supported the former explanation. Tetraploid *F. cymosum* might have originated independently in each of the diverged two regions, and the morphological difference between Tibet-Himalayan group and Yunnan-Sichuan group may be entirely dependent on the origin of these two groups.

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Evaluation of Plant Type Variation in Common Buckwheat in the northern region of Japan

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ABSTRACT

Variation of plant type in common buckwheat (*Fagopyrum esculentum* Moench) was analyzed by principal component analysis (PCA). Fifteen characters were measured; largest leaf area, plant height, length of main stem, length of eight internodes from the ground surface, number of nodes on main stem, number of branches, diameter of main stem and number of nodes on the lowest branch. The results of PCA showed different plant types from each country. Japanese varieties were of a type which had lower internode elongation and large plants. Chinese varieties had similar internode elongation and a small size of plant. French varieties had upper internode elongation and were of a mid size plant type. Nepalese varieties had similar internode elongation and were of a large size of plant type. Polish varieties had upper internode elongation and small size of plant type. Russian varieties had both lower and upper internode elongation and were mid size of plant type. Slovenian varieties had lower internodes elongation and a small size of plant type. Canadian varieties were not clear for elongation but had a mid size of plant type. As a result of cluster analysis using the Ward method, almost all Japanese varieties were separated from the European and Chinese varieties. The latter varieties were further divided into European and Chinese sub-groups.

INTRODUCTION

Common buckwheat (*Fagopyrum esculentum* Moench) is cultivated around the world. It is an important ingredient in many traditional recipes; for example, Russian kasha, French crepe, Italian pasta, Slovenian zganci, Chinese mian, Korean naengmyon and Japanese soba noodles which is one of the most popular noodles in Japan (Ujihara, 1982; Komeichi et al., 1992; Kreft, 1992). Soba is recognized as a healthy and natural food, the consumption of which is stable in Japan. To meet demand, the majority of buckwheat groats are imported from China, the US and Canada, and only 20% are produced domestically. Japanese millers and consumers, however, consider that domestic buckwheat is better quality than imported ones and as a result, the price of domestic buckwheat is three times higher than imported buckwheat.

As buckwheat productivity is low, buckwheat production is therefore less profitable for Japanese farmers than for other main crops such as rice or wheat. The cultivated area of buckwheat is, however, now increasing in Japan, because other major crop production has been restricted, therefore buckwheat cultivation has become to be fascinating for Japanese farmers. Recently two new common buckwheat varieties, 'Kitawasesoba' and 'Kitayuki', were bred and released in Hokkaido, which is the No. 1 buckwheat production area in Japan (Inuyama et al., 1995; Honda et al., 1995). With the increase in cultivated area in Japan, Japanese farmers require new, stable and high yielding varieties. These varieties, however, were not enough to increase the yield very much. Plant type is a very important character which is related to lodging resistance and upright plant standing in the field, and as a result it usually brings stable growth, easy management and high yield. Plant type consists of multiple characters including plant height, internode length, branching number etc. It is required to understand the tendencies of plant type at a glance in a simple figure. PCA is a useful method for the interpretation of traits from multiple characters like plant type. In this paper we report on 52 varieties that were analyzed and discuss the usage of PCA and plant type in relation to yield.

MATERIALS AND METHODS

Plant Materials

Fifty two varieties were utilized in this experiment (Table 1); 22 Japanese, four Canadian, six Russian, three Slovenian, five Polish, seven French, four Chinese and one Nepalese. The reason why the varieties tested were selected was as follows. Canadian varieties were the major varieties in the North America, these varieties have

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Table 1. Examined common buckwheat varieties.

ID No.	Variety	Ecotype	Origin	Notes
1	Kitawasesoba	Summer	Hokkaido, Japan	HNAES
2	Kitayuki	Summer	Hokkaido, Japan	HNAES
3	Botansoba	Summer	Hokkaido, Japan	Hokkaido GRC
4	Shimokawa	Summer	Hokkaido, Japan	Local variety
5	Kitami No. 3	Summer	Hokkaido, Japan	Local variety
6	Bihoro	Summer	Hokkaido, Japan	Local variety
7	Tanno/Hiushinai	Summer	Hokkaido, Japan	Local variety
8	Takinoue	Summer	Hokkaido, Japan	Local variety
9	Touya	Summer	Hokkaido, Japan	Local variety
10	Abuta	Summer	Hokkaido, Japan	Local variety
11	Shintoku	Summer	Hokkaido, Japan	Local variety
12	Shikaoi No. 1	Summer	Hokkaido, Japan	Local variety
13	Shikaoi No. 2	Summer	Hokkaido, Japan	Local variety
14	Botansoba (Furano)	Summer	Hokkaido, Japan	Tokachi AES
15	Tsubetsu	Summer	Hokkaido, Japan	Tokachi AES
16	Hashikamiwase	Mid-summer	Aomori, Japan	Aomori UHES
17	Ichinohe No. 2	Summer	Iwate, Japan	TNAES
18	Shinanonatsusoba	Summer	Nagano, Japan	Chushin AES
19	Chushinkei 7	Summer	Nagano, Japan	Chushin AES
20	Shinano No. 1	Mid-autumn	Nagano, Japan	Chushin AES
21	Kyushuakisoba	Autumn	Miyazaki, Japan	TNAES
22	Miyazakizairai	Autumn	Miyazaki, Japan	Miyazaki Univ.
23	Mancan	Summer	Canada	Morden AES
24	Tokyo	Mid-summer	Canada	Morden AES
25	CM-17	Summer	Canada	Morden AES
26	Tempest	Summer	Canada	Morden AES
27	Bogatyr'	Summer	Russia	Vavirov Institue
28	Skorospelaya 81	Summer	Russia	Vavirov Institue
29	Krasnostreletskaya	Summer	Russia	Vavirov Institue
30	Gloriya	Summer	Russia	Vavirov Institue
31	Maiskaya	Summer	Russia	Vavirov Institue
32	Yubileinaya 2	Summer	Russia	Vavirov Institue
33	Hruszowska	Summer	Poland	Shinshu Univ.
34	Emken	Summer	Poland	Shinshu Univ.
35	Pulawska 1	Summer	Poland	Shinshu Univ.
36	Pulawska 2	Summer	Poland	Shinshu Univ.
37	Pulawska 3	Summer	Poland	Shinshu Univ.
38	Crna Ajda	Summer	Slovenia	Shinshu Univ.
39	Bednia	Summer	Slovenia	Shinshu Univ.
40	Siva Dolensjka	Summer	Slovenia	Shinshu Univ.
41	Le Heller	Mid-summer	France	Shinshu Univ.
42	Saint Seglen	Summer	France	Shinshu Univ.
43	Monteneuf	Summer	France	Shinshu Univ.
44	Sevignac	Summer	France	Shinshu Univ.
45	Ruffiac	Summer	France	Shinshu Univ.
46	Pontivy	Summer	France	Shinshu Univ.
47	Gruel	Summer	France	Shinshu Univ.
48	Shandong	Summer	China	Shinshu Univ.
49	Yunnan South	Autumn	China	Shinshu Univ.
50	Hubei	Summer	China	Shinshu Univ.
51	Yunnan imports	Autumn	China	Shinshu Univ.
52	Simikot	Autumn	Nepal	Shinshu Univ.

been often used in Canadian experiments (Gubbels, 1977; Gubbels and Campbel, 1985). Polish and Slovenian varieties were also the major varieties in both countries, and they had been used many times in buckwheat experiments (Kubiczek, 1986; Kajfez-Bogataj and Gaberscik, 1986). Japanese varieties were also major varieties and Japanese local varieties were collected in Hokkaido district. All Russian varieties were representative varieties in Russia (Adachi, 1991). Chinese and Nepalese varieties were selected from another experiment (Ujihara, 1983), therefore it was assumed that these varieties were representative of local or major varieties in all countries. There were no varieties used from France, but French local varieties were collected in the district that buckwheat was cultivated.

The varieties were sown on June 11th, 1987 in an experimental field at the Hokkaido National Agriculture Experimental Station; 4.8 m^2 plot size, 66.7 plants/m^2 plant density, 60 cm row spacing with 2 replications. The fertilizer applied was 2-7-6 N-P_2O_2-K_2O (g/m^2).

Measured characters

The followed fifteen characters were measured to record the plant type for ten plants of each variety; largest leaf area (LA), plant height (PH), main stem length (LMS), length of cotyledon node from ground surface (LCO), first to seventh internode length from cotyledon node (LIN1–7), number of nodes on main stem (NN), diameter of main stem (DMS) and node number on the lowest branch (LN). The relationship between plant height and the length of internodes was evaluated (Fig. 1). Flowering time and maturing time were observed in the growing process. The number of flower clusters, yield per 10a and thousand seed weight were measured as the yield components.

Statistical analyses

Principal component analysis (PCA) and cluster analysis were executed on the personal computer. The computer software used in this experiment was *Excel statistics 2000 for Windows* by SSRI Ltd. Co. 1999, Tokyo, Japan.

RESULTS

The mean value and the standard deviation of fifteen characters related with plant type are shown in Table 2 for each country. The values of the standard deviation in all varieties were larger than the values of each country except for leaf area, plant height and main stem length of Polish varieties and the leaf area in Canadian varieties. The variation in each country was usually lower than that of all varieties. The correlation matrix of the fifteen characters is shown in Table 3. Plant height had a high positive correlation with the length of main stem, number of internodes and diameter of the main stem. Although each internode length on the main stem had a high correlation with both neighboring internodes, plant height had a higher correlation with the upper internodes than with the lower ones.

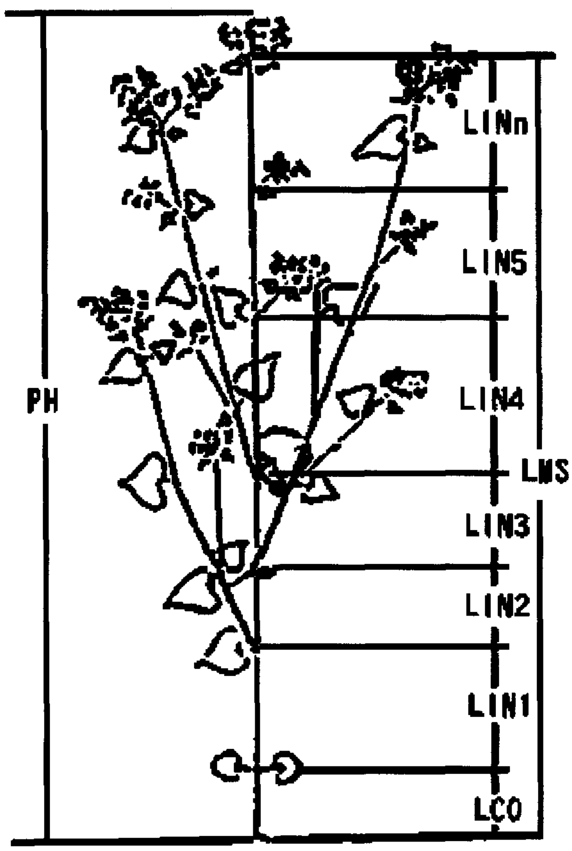

Fig. 1. Measured internode characteristics in relation to plant type.
Modified figure from Agriculture, Forestry and Fisheries Technical Information Society (1981).

These fifteen characters in a correlation matrix were analyzed by PCA. The eigen vector, factor loadings of three Z-scores, eigen value and cumulative contribution from PCA are shown (Table 4). The cumulative contribution in Z_1, Z_2 and Z_3 was 79.2%. These three Z-scores' values in each variety are shown (Table 5). Z_1 correlated with all fifteen characters, therefore Z_1 axis represented the size factor of the buckwheat plant. Z_2 had a positive correlation with lower internode length (LCO, LIN1–3) and negative correlations with upper internode length (LIN6 and 7), therefore the Z_2 axis presented the relative growth of the upper internodes length to the lower. Furthermore Z_3 had a positive correlation with upper internode length, therefore Z_3 axis represented the growth of the upper internodes.

The scatter diagrams of the relationship between Z_1 and Z_2 scores are shown (Fig. 2). Most of the Japanese varieties were located in the first quadrant, therefore they were of lower internodes elongation and large size of plant

Table 2. Mean value and standard deviation in all varieties and in each country.

Caracter	LA cm²	PH cm	LMS cm	LCO cm	LIN1 cm	LIN2 cm	LIN3 cm	LIN4 cm	LIN5 cm	LIN6 cm	LIN7 cm	NN No	NBR No	DST cm	LN No
(All varieties)															
Mean value	43.6	122.5	117.8	4.7	11.6	13.4	13.9	13.3	12.4	10.7	9.3	14.1	3.8	0.7	3.0
SD	10.73	16.95	17.42	1.20	2.64	1.95	1.80	1.65	1.29	1.20	1.30	2.29	0.74	0.06	0.78
(Japan)															
Mean value	49.7	129.0	125.5	5.7	13.3	14.5	15.0	14.4	13.1	10.8	9.2	14.5	4.2	0.7	3.6
SD	6.04	11.93	13.35	0.82	1.87	1.77	1.62	1.53	1.00	0.70	0.67	1.87	0.60	0.03	0.52
(Canada)															
Mean value	46.2	123.3	118.6	3.6	10.8	12.2	13.0	13.1	12.0	9.7	8.4	15.2	3.8	0.7	3.2
SD	11.95	6.32	6.35	0.70	0.54	0.89	0.49	0.85	1.30	1.08	0.79	1.03	0.55	0.04	0.25
(China)															
Mean value	32.1	125.2	113.9	3.7	8.5	11.6	12.1	11.4	10.3	9.7	8.3	16.5	4.3	0.7	2.1
SD	4.87	10.17	8.30	0.51	1.49	1.43	1.76	0.66	0.57	0.66	1.07	1.13	0.44	0.03	0.52
(France)															
Mean value	44.9	125.9	121.5	3.3	8.7	12.5	13.4	13.2	12.7	12.2	11.3	13.6	3.0	0.7	3.2
SD	9.76	10.95	11.58	0.30	1.69	1.34	1.14	0.67	0.38	0.49	0.59	0.77	0.21	0.04	0.24
(Nepal*)															
Mean value	53.7	175.0	166.8	3.3	12.9	15.0	15.6	15.5	13.0	11.0	9.7	21.5	5.3	0.9	3.8
SD	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
(Poland)															
Mean value	39.2	105.4	100.5	4.3	10.0	11.8	12.2	11.7	11.3	10.2	9.0	13.0	3.5	0.7	2.2
SD	11.65	18.58	18.71	0.72	2.55	1.88	1.47	1.54	1.20	1.14	1.48	1.36	0.52	0.09	0.37
(Russia)															
Mean value	36.5	110.7	106.7	4.9	12.7	13.9	13.9	13.1	12.6	10.9	9.0	11.8	3.1	0.7	2.3
SD	5.19	6.83	8.17	0.98	2.46	1.36	1.30	0.56	0.73	1.42	1.63	1.70	0.34	0.02	0.20
(Slovenia)															
Mean value	26.0	96.4	92.0	4.7	11.4	12.9	13.2	11.5	10.7	9.3	8.4	11.0	3.3	0.6	2.0
SD	7.93	8.67	9.01	0.81	0.51	1.38	0.59	0.84	0.49	1.31	0.90	1.14	0.17	0.06	0.50

*: N=1

LA: Leaf area, PH: Plant height, LMS: main stem length, LCO: Length of cotyledon node from ground surface, LIN1: 1st internode, LIN2: 2nd internode, LIN3: 3rd internode, LIN4: 4th internode, LIN5: 5th internode, LIN6: 6th internode, LIN7: 7th internode, NN: Number of nodes on main-stem, NBR: Number of branches, DST: Diameter of main stem, LN: Number of nodes with lowest branch.

type. The Chinese varieties were located in the third quadrant, therefore they were not of lower internodes elongation, but had a small sized type of plant. The French varieties were located in the third and forth quadrants, therefore they did not have lower internodes elongation but had a mid size of plant type. The Nepalese varieties were located in the fourth quadrant, therefore it was not the lower internodes elongation but the large size of plant type. Polish varieties located in both of second and third quadrants, therefore they were not the lower internodes elongation but the small size of plant type. Almost all Russian varieties located near in Z_1='zero' of the second quadrant, therefore they were the lower internodes elongation and mid size of plant type. Slovenian varieties located in the second quadrant, therefore they were the lower internodes elongation and small size of plant type. Canadian varieties, however, located in near Z_1='zero' of three quadrants; for example, 'Mancan' in the first quadrant, 'Tokyo' in the second quadrant, 'Tempest' and 'CM-17' in the third quadrant, therefore they were not clear about internodes elongation but were the mid size of plant type.

The scatter diagrams of relationship between Z_1 and Z_3 scores are shown in Fig. 3. Most of Japanese varieties located in the fourth quadrant, therefore they were not the upper internodes elongation but the large size of plant type. Chinese varieties located in the third quadrant, therefore they were not the upper internodes elongation type but the small size of plant type. French varieties located in the first and second quadrants, therefore they were the upper internodes elongation and mid size of type. The Nepalese variety was located in the fourth quadrant, therefore it did not have upper internodes elongation but had a large size of plant type. Most of the Polish varieties were located in the second quadrants, therefore they had upper internodes elongation and a small size of plant type. Most of Russian varieties were located in or near Z_1='zero' of the second quadrant, therefore

Table 3. Correlation matrix of fifteen characters in related to plant type.

Character	LA	PH	LMS	LCO	LIN1	LIN2	LIN3	LIN4	LIN5	LIN6	LIN7	NN	NBR	DST	LN
LA	1.0000														
PH	0.5311**	1.0000													
LMS	0.5747**	0.9812**	1.0000												
LCO	0.2346	0.0433	0.0961	1.0000											
LIN1	0.3516*	0.1857	0.2597	0.8385**	1.0000										
LIN2	0.4145**	0.3031*	0.3847	0.6895**	0.8694**	1.0000									
LIN3	0.3709**	0.3322*	0.4207**	0.5769**	0.7477**	0.8787**	1.0000								
LIN4	0.5040**	0.5485**	0.6238**	0.4180**	0.5754**	0.6912**	0.8049**	1.0000							
LIN5	0.5635**	0.4338**	0.4889**	0.3592**	0.3942**	0.4638**	0.4513**	0.7319**	1.0000						
LIN6	0.3832**	0.4646**	0.4957**	−0.0134	−0.0102	0.1798	0.0852	0.2937*	0.6279**	1.0000					
LIN7	0.3244*	0.4940**	0.5039**	−0.1881	−0.1935	0.0179	−0.0094	0.1835	0.4037**	0.8423**	1.0000				
NIN	0.2766*	0.8307**	0.7836**	−0.2298	−0.1460	−0.0881	−0.0138	0.2016	0.0679	0.1550	0.2333	1.0000			
NBR	0.3664**	0.5400**	0.4964**	0.1325	0.1106	−0.0158	0.0013	0.1614	0.1280	−0.0561	−0.1044	0.6639**	1.0000		
DST	0.6077**	0.7715**	0.7544**	0.0332	0.2040	0.3621**	0.3638**	0.5133**	0.4880**	0.5345**	0.5146**	0.5130**	0.3137*	1.0000	
LBR	0.5904**	0.5906**	0.6581**	0.3791**	0.4141**	0.4830**	0.5990**	0.6766**	0.5206**	0.3414*	0.2661	0.3494*	0.2409	0.4768**	1.0000

*, **: Significant at 5% and 1% levels, respectively. Same characters in Table 2.

Table 4. Eigen vector, factor loading, eigen value and cumulative contribution extracted from PCA of 15 characters for 52 varieties.

	Eigen vector			Factor loading		
Character	Z_1	Z_2	Z_3	Z_1	Z_2	Z_3
LA	0.2813	−0.0405	0.0079	0.7287**	−0.0736	0.0107
PH	0.3173	−0.2549	−0.1592	0.8219**	−0.4633**	−0.2174
LMS	0.3370	−0.2125	−0.1244	0.8728**	−0.3861**	−0.1699
LCO	0.1646	0.3993	−0.0777	0.4264**	0.7255**	−0.1061
LIN1	0.2196	0.4018	−0.1017	0.5689**	0.7301**	−0.1389
LIN2	0.2652	0.3434	0.0339	0.6868**	0.6240**	0.0463
LIN3	0.2688	0.3153	−0.0161	0.6962**	0.5729**	−0.0219
LIN4	0.3219	0.1484	0.0243	0.8337**	0.2697	0.0332
LIN5	0.2835	0.0463	0.2703	0.7343**	0.0842	0.3690**
LIN6	0.2074	−0.2048	0.4856	0.5372**	−0.3721	0.6630**
LIN7	0.1673	−0.2944	0.4598	0.4334**	−0.5349**	0.6277**
NN	0.1785	−0.3641	−0.5222	0.4622**	−0.6617**	−0.5195**
NBR	0.1449	−0.1681	−0.5222	0.3754**	−0.3055*	−0.7130**
DMS	0.2972	−0.1881	0.0422	0.7698**	−0.3418*	0.0576
LN	0.3043	0.0335	−0.0340	0.7882**	0.0608	−0.0465
Eigen value				6.71	3.20	1.86
Cumulative contribution (%)				44.7	66.9	79.2

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

Table 5. Z-scores in the examined common buckwheat.

ID No	Z_1	Z_2	Z_3
1	0.0309	1.5884	−0.2260
2	1.4430	1.1632	−1.3761
3	2.0316	1.8346	−0.7349
4	2.6244	−0.8611	−0.6399
5	1.4991	−0.3211	−0.4311
6	2.0204	0.6689	−0.0523
7	1.6098	2.0192	0.6877
8	3.2607	−0.0002	0.6192
9	1.3430	−0.0502	−0.9902
10	1.8861	1.5676	−0.7363
11	3.2812	1.5645	−0.0721
12	4.4218	0.6356	−0.4795
13	3.5328	0.4757	−0.0351
14	1.1462	0.8098	−0.6233
15	0.9549	−0.4354	−0.2790
16	1.5263	2.3865	−0.2837
17	−3.2205	−2.3776	−1.2377
18	0.1060	4.0646	−0.0737
19	2.8143	3.7027	0.9173
20	3.5341	−0.9298	0.1106
21	1.9338	−1.6555	−2.1677
22	1.3377	−0.6902	−1.6558
23	0.3200	0.5636	−0.6082
24	−1.9410	0.0163	−1.5672
25	−0.0343	−2.1651	−2.0696
26	−0.8254	−1.5359	0.6166
27	−0.5911	0.8720	1.5566
28	−2.8214	3.7457	−0.9758
29	−0.4679	2.3415	1.3596
30	−0.4645	−0.6911	1.6139
31	−1.4118	2.8072	1.7831
32	−1.2263	−1.6881	0.9300
33	−0.1778	−0.2116	0.6450
34	−1.6015	0.7650	1.0014
35	−2.4784	−1.4257	0.3272
36	−0.6865	−0.6658	−0.4460
37	−8.7208	0.4114	−0.4565
38	−6.0615	2.5491	−0.9423
39	−3.2548	1.6254	0.6375
40	−2.6735	0.6093	0.4792
41	4.0095	−1.9093	2.0201
42	−0.5980	−2.4409	2.7138
43	−0.1778	−1.4649	2.4777
44	−1.0409	−2.3399	1.8824
45	−0.4677	−1.8707	2.4525
46	−0.7182	−1.2727	2.8062
47	−0.0625	−1.8383	1.3225
48	−2.5047	−0.1466	−2.5738
49	−2.6629	−0.5133	−2.1611
50	−2.1131	−2.5592	−1.5539
51	−2.8187	−3.6975	−0.9200
52	5.1564	−3.0353	−2.5913

Same ID-No. as in Table 1.

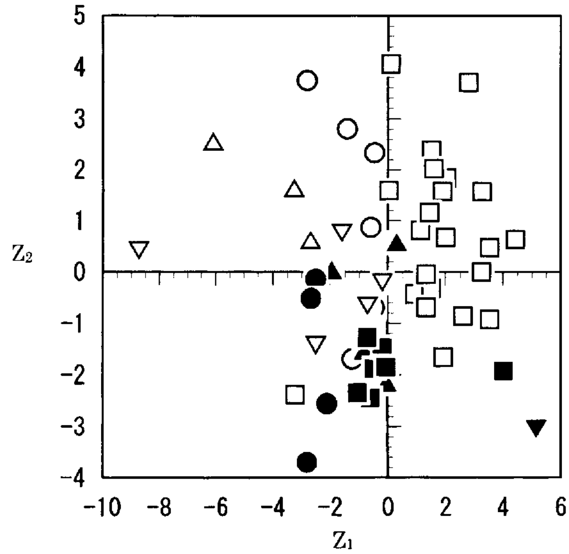

Fig. 2. Scatter diagram for 52 varieties based on 2 component scores of Z_1 and Z_2.
□ : Japan, ▲ : Canada, ● : China, ■ : France, ▼ : Nepal, ▽ : Poland, ○ : Russia, △ : Slovenia.

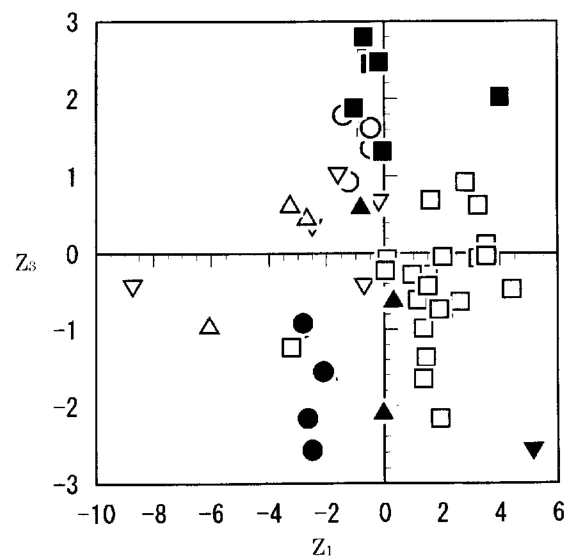

Fig. 3. Scatter diagram for 52 varieties based on 2 component scores of Z_1 and Z_3.
Same symbols in Fig. 2.

Table 6. Relationship between Z-scores and agronomic characters

Z-scores	Flowering time	Maturing time	Number of flower cluster	Yield	Thousand seed weight
Z_1	0.5788**	0.5191**	−0.1160	0.1735	0.2492
Z_2	−0.2395	−0.4575**	−0.6588**	0.1554	0.6036**
Z_3	−0.3299*	−0.5146**	−0.5644**	0.5253**	−0.1326

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

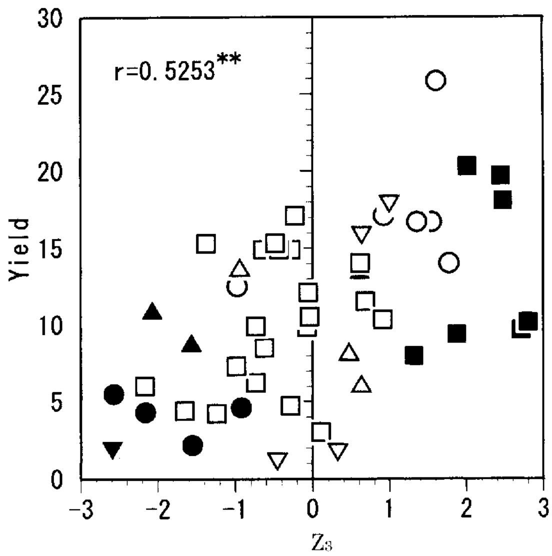

Fig. 4. Relationship between Z_3 scores and yield.
Same symbols in Fig. 2.

they were had upper internodes elongation and a mid size of plant type. The Slovenian varieties located in the second and third quadrants, therefore they did not have upper internodes elongation but had a small size of plant type. The Canadian varieties, however, were located in three quadrants; for example, 'Mancan' was in the forth quadrant, 'Tokyo' and 'CM-17' in the third quadrant and 'Tempest' in the second quadrant, therefore they were not clear about internodes elongation in this figure, but had a mid size of plant type.

The relationship between Z-scores and the important agronomic characters is shown in Table 6. Z_1 had the significant positive correlations with flowering time and maturation time. Z_2 had a significant negative correlation with maturation time and the number of flower clusters and a positive correlation with thousand seed weight. Z_3 had a significant negative correlation with maturation time and the number of flower clusters and a significant positive correlation with yield.

The relationship between the Z_3 score and yield is shown in Fig. 4. The Japanese varieties were located in the mid area in this graph. The Russian and French varieties, which were of a upper internodes elongation type, showed their high yield performance because of being located in the upper right side. In contrast the Chinese, Nepalese, Polish and some of the Japanese varieties showed low yield by being located in the lower left side.

A dendrogram was constructed by cluster analysis with using the Ward method on the basis of Euclid distance in Z_1, Z_2 and Z_3 scores (Fig. 5). The varieties used in this experiment were divided into two groups, a Japanese group and European/Chinese group which was divided further into an European sub-group and a Chinese sub-

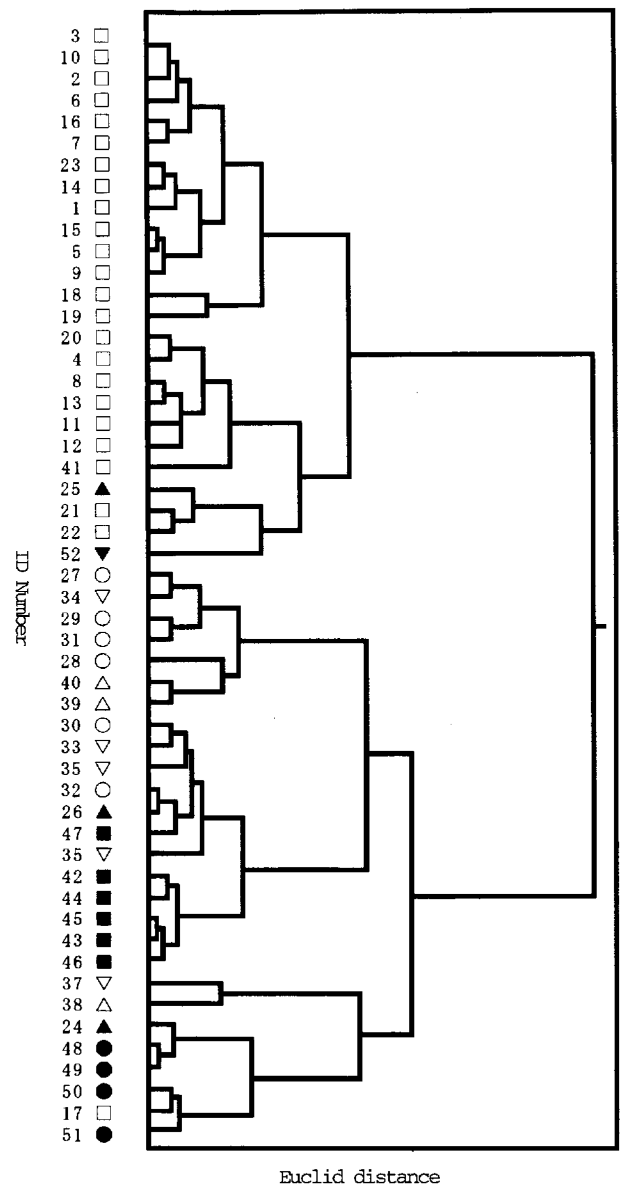

Fig. 5. Dendrogram constructed by cluster analysis for 52 varieties based on three component scores of Z_1, Z_2 and Z_3.
Same ID-No. in Table 1. Same symbols in Fig. 2.

group. The European sub-group was further divided into several minor-groups of each country.

DISCUSSION

Plant height had a higher positive correlation with upper internode elongation than with lower internodes on the main stem (Table 2). Plant height seemed to be decided by upper internode elongation during the later growth phase. Z_1 had a high correlation with all characters measured in this experiments, therefore it meant the size factor as shown in Table 4. The cumulative contribution in Z_1, Z_2 and Z_3 scores showed a high value near 80%, therefore these three scores seemed to represent most of the buckwheat plant types.

Table 7. Plant type in varieties in each countries from Z_1, Z_2 and Z_3 scores.

Country	Elongation-type*	Whole size of plant	Notes
Japan	Lower	Large	
Canada	—	Mid	Various origins
China	Same	Small	
France	Upper	Mid	
Nepal	Same	Large	
Poland	Lower	Small	
Russia	Lower/Upper	Mid	
Slovenia	Lower	Small	

*: Lower; Lower-internode elongation type, Upper; Upper-internode elongation type. Same; Same internode elongation type.

The tendency of the plant type was divided into each country from Z_1, Z_2 and Z_3 by the PCA results (Table 7). The Z_1 value decided the size of the plants, and the Z_2 and Z_3 values decided the elongation type in the internodes. The Japanese varieties seemed to belong to a plant type that has a large size and elongates in the lower internodes. The Chinese varieties seemed to belong to a plant type that had a the small size and elongates by the same degree in each internode. The French varieties seemed to belong to a plant type that has mid size and elongates in the upper internodes. The Nepalese variety seemed to belong to a plant type that has large size and elongates by same degree in each internode. The Polish varieties seemed to belong to a plant type that has small size and elongates in the upper internodes. The Russian varieties seemed to belong to the plant type that had a mid size and elongates in both of the lower and upper internodes. The Slovenian varieties seemed to belong to a plant type that has a small type and elongates in the lower internodes. In contrast the Canadian varieties seemed to be not clear in their tendency; for example 'Mancan' was very similar to the Japanese varieties, 'Tempest' was similar with Polish or French varieties, and 'Tokyo' was similar with Russian or Slovenian varieties. It was guessed that Canadian genetic resources consisted of various resources derived from Japan or East Europe.

Tested varieties were mainly divided in two group, Japanese and European/Chinese types. European/Chinese group were further divided into a Europe sub group and a Chinese sub-group from the contracted dendrogram (Fig. 5). The European sub-group was divided into minor groups of each country. In contrast the Canadian varieties were seen to be distributed in many groups including the Japanese and the European group. It also was assumed that the Canadian varieties were bred from various resources derived from Japan and other countries.

Japanese group seemed to locate in special group from this dendrogram, therefore the plant type of the Japanese varieties were different from other countries' varieties except for the Canadian variety, 'Mancan'. Japan is an island country, therefore it played the role of being isolated by the sea, and would seem that Japanese varieties uniquely evolved in this status of isolation. 'Mancan' was developed for the Japanese buckwheat market and some of its breeding materials may have been derived from Japanese genetic resources.

The typical traits of summer and autumn types of Japanese buckwheat have been classified by PCA (Ohsawa and Tsutsumi, 1994; Tetsuka and Uchino, 2002). The materials they used were only Japanese varieties, so their results were clear for the Japanese cropping system, but their results were only for the Japanese group in common buckwheat and did not evaluate other varieties in the world. Ohsawa and Tsutsumi (1994) used not only characters that were related with plant type or growth habit such as flowering time, but also yield or seed traits such as test weight. Their Z-scores axis included growth habit, plant traits, yield ability, and therefore it was useful to compare among the Z-scores. But each Z-score didn't mean a specific traits such as plant type, therefore, they were not enough to explain the focused plant type. In contrast Tetsuka and Uchino (2002) focused on plant type, but they measured only the first internode length and not the other internodes length. Plant height is the sum of all internode lengths and one of the most important parts which influences the management of cultivation or the yielding ability of the crop. Their Z-scores were not completely enough about plant height in plant type. Internode elongation contributed to the size of the plant type, plant height and yield, therefore analysis of internode elongation in plant type was considered very important for improving yield from the plant type in buckwheat breeding.

The buckwheat plant type was also analyzed by using computer imaging (Minami et al., 1991). This method was very easy for us to understand the image of plant type. The analysis used the dimensional posture of the plant, as well as the silhouette area of the buckwheat to analyze plant type. It was a useful technique when comparing a few varieties, because the imaging file usually utilized a major portion of the capacity of the hard disk of the computer. Therefore, imaging analysis was not effective for estimating many varieties. It is considered important to be able to show a lot of characters of many varieties at a glance in an only figure.

The semi-dwarf trait has been considered as an important character in many crops, because it is related with the lodging resistance and high yield. The buckwheat stem is weak to strong winds and plant lodging decreases the yield. Lodging resistance is therefore an important character for stable and high yield in buckwheat. It appears that the size of plant was usually decided by internode elongation in common buckwheat. French varieties seemed to have upper internode elongation and showed low elongation in the lower internodes, and Z_3 which related with upper elongation of the internodes had a high positive correlation with yielding ability (Table 6). The French varieties had a high Z_3 value and high yield (Fig. 4). It is considered important for breeding of a Japanese variety to introduce the characteristics of the French varieties with a relatively short internode in lower part of the plant to improve lodging resistance.

We couldn't fully understand the tendency in plant type caused by a single character such as the plant height. For example, Both of Chinese and Slovenian varieties were only small for plant height. We can understand the difference of among the varieties in the original place by using not only the plant height but also the each internode length in the multiple characters. PCA seemed to be useful to clear the origin of the genetic resources and suggested ways to improve the plant type in developing high yielding varieties.

Japanese varieties seemed to belong to a specific group in the world from the viewpoint of plant type. The main reason may be related to the isolation of genetic resources in Japanese history or the geographical islands. We should investigate other characters, especially groat quality, in varieties from around the world to evaluate the variable characteristics which may be used for improving yield and seed quality for our next step.

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The incidence of imperfect flowers and its effect on fertilization in common buckwheat grown under water stress conditions

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Key words: common buckwheat, fertilization, imperfect flower, leaf temperature, water stress

ABSTRACT

The effect of water stress on the incidence of imperfect flowers in common buckwheat was studied on the basis of the concept of a module in relation to fertilization. There was a significant positive correlation between the number of bloomed flowers and that of imperfect flowers which is similar correlation as between the number of bloomed flowers per module and that of fertilized flowers per module as previously reported (Hagiwara et al., 2002). Furthermore, since the individual-based and module-based imperfect flowering rates were highly correlated, the imperfect flowering rate was expected to be well estimated by investigating only a limited number of modules on a plant. This would be of great help in facilitating the analysis of the effects of the incidence of imperfect flowers on fertilization. Soil water regime influenced the time course change in the imperfect flowering rate. Negative correlations between the temperature difference between leaf and air (TDLA) as a crop water stress index and the individual- and module-based imperfect flowering rates were found. The imperfect flowering rate and the fertilization rate of perfect flowers were not highly correlated, suggesting that the incidence of imperfect flowers had no or only a slight direct effect on the fertilization capability of the perfect flowers. The mechanisms that control the fertilization rate and the imperfect flowering rate are suggested to be different.

INTRODUCTION

In an attempt to improve the productivity of common buckwheat, a series of new analyses based on the concept of a module, which is the smallest repeated unit of development capable of producing daughter units or seeds through the activity of their founding meristems (Harper, 1977; Tuomi and Vuorisalo, 1989; White, 1979), has been recently carried out (Inoue et al., 1998; Inoue and Hagiwara, 1999). These studies demonstrated that a new analysis of the fertilization rate (number of fertilized flowers/number of bloomed flowers) based on a module concept seemed to provide a useful basis for the improvement of common buckwheat yield. In the studies based on the module concept, the fertilization rate was determined by investigating the numbers of bloomed and fertilized flowers in each module on a plant. The investigation of the fertilization rate on a module basis was similar to that on a flower cluster basis, but the former differs from the latter in the terminal and some of the large auxiliary flower clusters, because they consist of a number of modules, while most auxiliary flower clusters consist of only one module.

The fertilization rate in common buckwheat is often low and poor fertilization is widely recognized as one of the important causes of low productivity in common buckwheat. This report as well as our previous one (Hagiwara et al., 2002) deals with experiments to clarify the effects of water stress on the fertilization of common buckwheat. Our previous report (Hagiwara et al., 2002) showed that the fertilization rate based on a module concept was correlated with the temperature difference between leaf and air (TDLA) as a simplified indicator of crop water stress index (Idso et al., 1981; Jackson et al., 1981). TDLA is reported to be useful for the evaluation of the water stress in some crops (Fujii et al., 2000; Geiser et al., 1982). Since fertilization in common buckwheat is partly influenced by the frequent development of sterile flowers (imperfect flowers) mainly due to an abnormal pistil, the effect of water stress on the incidence of imperfect flowers and its effect on the fertilization rate in common buckwheat were studied.

MATERIALS AND METHODS

Experimental plots

This experiment was conducted in 2000, using a common buckwheat (*Fagopyrum esculentum* Moench) variety Shinano No. 1. Control plots of 100 cm×40 cm were established with four replications by planting Shinano No. 1 in the Research Farm of the Faculty of Agriculture, Shinshu University. Experimental plots for two water stress treatments each with four replications were prepared by burying plastic containers (53 cm×40.5 cm, and

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33.5 cm deep) into soil in a vinyl house about 30 m away from the control plots. Each replication consisted of one plastic container. Soil collected from the field was sieved and well air-dried before placing it into the containers. Seeding was conducted on July 28 with a row spacing of 10 cm and hill spacing of 10 cm in all plots. In the water stress treatments, the water supply was done just after seeding and whenever it is necessary until the 18th day after seeding, when the water stress treatment was initiated by limiting the water supply. The amount of water supplied from the 18th day after seeding in the two water stress treatments was 1L everyday (1L/1D) and 4L every four days (4L/4D), respectively. Fertilizers and agricultural chemicals were not applied to any of the plots. All experimental plots in the vinyl house were surrounded by Shinano No. 1 plants sown on the same day as that in experimental plots in the manner of row spacing of 70 cm and hill spacing of 10 cm. The vinyl house was covered with vinyl film only at the roof part to prevent excessive rise in air temperature in it and not to prevent pollinator attendances.

Fertilization rate

The fertilization rate was investigated on five plants from each treatment after harvest. The total number of bloomed flowers and that of fertilized flowers were counted for each module as previously reported (Inoue et al., 1998; Inoue and Hagiwara, 1999). When any growth of achene was visible, the flower was regarded as fertilized as similar to the previous reports.

In this report, we determined two fertilization rates, individual-based and module-based ones, as defined in our previous report (Hagiwara et al., 2002). The former is an ordinary fertilization rate of a plant and the latter is based on the linear regression between the number of bloomed flowers per module (N) and that of fertilized flowers per module (F). A significant linear regression $F=\alpha+\beta N$ has been reported for N and F in a plant (Inoue et al., 1998; Inoue and Hagiwara, 1999), where β is a parameter that shows the property of flower fertilization of a plant based on the concept of a module. $\beta \times 100$ was thus regarded as the fertilization rate based on a module concept.

Imperfect flowering rate

The imperfect flowering rate was investigated two or three times on five plants per replication during the early to mid flowering period. The procedures were as follows. The number of flowers that bloomed on the day of investigation (N') was counted for each module. Flowers with an abnormal pistil were regarded as imperfect flowers, and the number of those that bloomed on the day of investigation (I) was counted for each module. The rate of imperfect flowers of a plant on each investigation day was calculated by $I \times 100/N'$. The rate of imperfect flowers for a plant during the whole flowering period was estimated by [sum of I]$\times$100/[sum of N']. These rates are referred to as individual-based imperfect flowering rates in contrast to the module-based imperfect flowering rates described below.

Similar to the module-based fertilization rates, the module-based imperfect flowering rates were determined based on the linear regression between N' and I. A module-based imperfect flowering rate on each investigation day was defined as $\delta \times 100$, where δ was the regression coefficient of the linear regression $I=\gamma+\delta N'$. A module-based imperfect flowering rate for the whole flowering period was estimated by $\delta' \times 100$, where δ' was the regression coefficient of the linear regression [sum of I] $=\gamma'+\delta'$ [sum of N'].

Leaf and air temperature

The leaf temperature was recorded on 10 leaves for each treatment using a non-contact, thermal-radiation-type thermometer (Minolta 505S, Minolta, Tokyo) during 12:00–14:00 on clear days with no or only light wind during the early to mid flowering period. Leaf temperature was measured for the uppermost or the second uppermost fully expanded leaf on the main stem. However, the data was collected for the largest and youngest leaf on a main stem when the two leaves were too small to be measured. Air temperature was simultaneously recorded with leaf temperature to determine the temperature difference between leaf and air (TDLA: leaf temperature minus air temperature) of each leaf.

RESULTS

Fertilization rate

We have already reported the details on the effect of water stress on fertilization rate observed in similar experiments as described in this report (Hagiwara et al., 2002). Since the present report includes the analysis of the relationship between fertilization rate and imperfect flowering rate, only the major results of our previous report are briefly described. The individual-based fertilization rate was not significantly influenced by water stress. On the other hand, the module-based fertilization rate was influenced by water stress and was correlated with TDLA, showing that the module-based fertilization rate responded more sensitively to water stress than the individual-based fertilization rate.

Imperfect flowering rate

Time course changes in individual-based imperfect flowering rates were different between control and water stressed treatments (Fig. 1). The individual-based imper-

also be a useful way of yield analysis as reported for common buckwheat grown under normal conditions (Inoue et al., 1998; Inoue and Hagiwara, 1999). Our previous report (Hagiwara et al., 2002) demonstrated that the analysis based on a concept of a module was useful, as the module-based rather than the individual-based fertilization rate correlated better with the degree of water stress (TDLA).

The application of the analysis of the rate of imperfect flowers based on a concept of a module was attempted in this report. Since imperfect flowers are not capable of being fertilized because of the abnormality in their pistils, the incidence of imperfect flowers is recognized as one of the potential factors leading to poor fertilization. Fig. 1 showed a clear difference in the incidence pattern of imperfect flowers among the treatments. In addition, individual- and module-based imperfect flowering rates responded well to TDLA. These results showed that the incidence of imperfect flowers was influenced by water stress. The result that the correlation between the module-based imperfect flowering rate and TDLA was slightly higher than that between the individual-based imperfect flowering rate and TDLA seemed similar to the higher correlation between the module-based fertilization rate and TDLA than that between the individual-based fertilization rate and TDLA as found by Hagiwara et al. (2002). This again suggested that an application of a concept of a module is effective in the analysis of the relationship between water stress and the process related to the yielding in common buckwheat.

It is certain that the incidence of imperfect flowers has negative effect on fertilization. However, correlation between imperfect flowering rate and fertilization rate of perfect flowers was not significant when they were individual-based (Fig. 5) and was significant but weak when module-based (Fig. 6). This implied that the incidence of imperfect flowers affected the fertilization rate simply by reducing the ratio of the number of flowers capable of fertilization to that of total flowers and that it had no or only a slight direct effect on the fertilization capability of the perfect flowers. A negative correlation between TDLA as an index of the degree of water stress and the imperfect flowering rate (Figs. 3 and 4) in contrast to the positive correlation between TDLA and the fertilization rate (Hagiwara et al., 2002) suggests that the effect of water stress, in other words, the mechanisms that control the fertilization rate and the imperfect flowering rate are different. Further detailed study is necessary to clarify the effect of water stress on the fertilization rate of perfect flowers and that on the incidence of imperfect flowers. However, such study would be laborious, because it requires the determination of the number of perfect flowers and that of imperfect flowers, which is obtainable by a careful observation of a pistil of each flower to identify whether or not it is abnormal. An efficient investigation technique is essential for further research to be proceeded. Inoue et al. (2002) reported that a good estimate of the fertilization rate of a plant was obtainable by investigating a limited number of flower clusters on a plant. Since a highly significant positive correlation was found between the module-based and individual-based imperfect flowering rates (Fig. 2), the investigation of imperfect flowers on a limited number of modules in a plant is expected to give a good estimate of the imperfect flowering rate of a plant. The facilitation of the analysis of the effect of water stress or other environmental stresses on the fertilization of common buckwheat seems promising by the observation of the numbers of total, imperfect and fertilized flowers on a limited number of modules on a plant.

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Effects of fertilization and inflorescence removal on photosynthesis and hay yield of buckwheat

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ABSTRACT

This study was conducted to determine the optimum conditions for production of high-yielding hay by increased fertilization and removal of the flower inflorescences before seed setting. The photosynthetic rate and leaf fresh weight of common buckwheat cultivars, Suwon No. 1 and No. 2 were slightly higher in plants of which the inflorescences were removed before flowering than in plants with intact inflorescences. Increased fertilization did not affect the photosynthetic rate, regardless of the presence of an inflorescence. Fresh leaf weight was higher in Suwon No. 2 than Suwon No. 1 and higher under standard fertilization than under an increased fertilization rate. Clfa 39, a Tartary buckwheat variety showed the highest assimilation rate, in the treatment of removed inflorescences under standard fertilization. Inflorescence removal did not increase the rutin content in buckwheat regardless of the fertilization level.

INTRODUCTION

Although buckwheat has been a traditional food crop, it has recently received increased attention as an important medicinal herb due to the discovery of the health benefits of buckwheat and its main constituents. Buckwheat has also been cultivated by farmers as a functional crop of which hay, including the leaves, stems and flowers, can be used as a health food or medicine (Park et al., 2001; Lee et al., 2001). Rutin has been found in both buckwheat grain and hay. Buckwheat has become a highly safe and healthy medicinal plant with an efficacy for vascular disorders caused by fragile or permeable capillaries (Campbell, 1998). Kwon (personal communication) has recently conducted a study on the prevention and cure of obesity by feeding swine buckwheat sprouts.

Rutin powder is purified from the extracts of buckwheat hay harvested at the flowering stage and commercialized among food or pharmacy companies in Japan (Matsumoto, 2000). All the vegetative organs of buckwheat, including the leaf, stem and the reproductive organs (the flower) are known to be a valuable source of rutin powder and other bioactive products. Thus, a high yield of buckwheat hay, including the inflorescences, is needed to increase the quantity of rutin powder.

In general, cultural practices of buckwheat plants have been mainly employed to increase the production of the grain. However, changes in cultural practices for buckwheat should be done, not only for the grain, but also for the hay which includes clusters of inflorescences.

The growth rate of plants and their organs during development is variable. The increase in plant biomass is not the same during all phases of plant growth. Therefore, it was thought that the production of buckwheat hay may be improved by controlling organ development, such as inflorescence removal.

The inflorescences of buckwheat plants appear to be cymose inflorescences which are made up of cymes. Each cyme presents a small bract at its base and forms up to five or six flowers. Flowers continue to appear within the inflorescences during an extended period of time. It is well known that there is a drastic reduction of grain number relative to the number of flowers formed (Halbrecq and Ledent, 2001).

Our final goal is to breed a new variety and to improve cultural practices for high-yielding buckwheat hay. We have tried to determine the optimum condition for high-yielding hay by increased fertilizer application and by removal of the inflorescences before seed setting.

MATERIALS AND METHODS

Three cultivars of two species of buckwheat were used for this experiment; *Fagopyrum esculentum* (cv. Suwon No. 1 and Suwon No. 2). and *F. tartaricum* (cv. Clfa 39). This experiment was conducted in the field at the University Farm of Kangwon National University in March, 2002. Seeds of the three cultivars were sown at a spacing of 30 cm between rows and a density of 5 g per m^2.

The rates of fertilization were either standard fertilization (N-P-K 10-29-20 400 kg/10a) or increased fertilization (N-P-K 10-29-20 800 kg/10a) using a compound fertilizer for soybean. The size of each plot was 2 m×4 m. The inflorescences of half the number of plants in each plot were removed using a hand cutter at flowering stage. Twenty plants in each treatment were harvested and measured for fresh weight.

The photosynthetic rates of 10 leaves per plot for photosynthetic active radiation were measured 10 days after removing the inflorescences by using a portable photosynthesis system (LI-6400, LI-COR). The leaf position measured for photosynthetic rate was on the first flowering node. The rutin content in the leaves sampled from each treatment were measured using a standard method as described previously by Park et al. (2000).

RESULTS AND DISCUSSION

Photosynthesis of the three cultivars under different fertilization and inflorescence defoliation

Suwon No. 1 showed a higher photosynthetic rate and leaf fresh weight between the two cultivars of common buckwheat. The photosynthetic rate and leaf fresh weight of Suwon No. 1 and No. 2 were slightly higher in plants from which the inflorescences were removed (inflorescence cutting) than in complete plants having no flower removal (inflorescence intact). Increased fertilization did not increase the photosynthetic rates regardless of inflorescence removal.

On the other hand, Clfa 39 which is a Tartary buckwheat, showed the highest assimilation rate in the treatment of inflorescence removal and under standard fertilization. However, evaporation in Clfa 39 was higher in the non-removal treatment and under the increased fertilization, as shown in Table 1.

In buckwheat the partitioning of assimilates to different organs, especially the partitioning of dry matter into grain and vegetative parts is of great importance (Kajfez-Bogataj, 1987). Leaves of buckwheat reached maximum weight before the end of the flowering period, after which most of the photosynthates were probably used for grain production while the weight of the stem increased throughout the growing period (Kajfez-Bogataj and Knavs, 1985).

The effect of fertilizer on plant growth is a key point for improving stable and high productivity of buckwheat hay. Table 1 shows that all of the measured values are greater under standard fertilization than under increased fertilization. These results are similar to Hayashi's finding (2001) that nitrogen and phosphorus had a larger effect on dry matter production (seed yield characteristics) than on plant growth. The light saturation point for leaf photosynthetic rate of buckwheat in PK, NK, NP and NPK plots at ripening period was 50–60% higher than in non-fertilizing plots (Hayashi, 2001). Growth on drill seeding has been reported to be better under increased fertilization (N-P2O5-K2O: 80-60-80 kg/ha) than under standard fertilization (Noh et al., 2001).

Assimilation rates in this experiment were higher when the amount of fertilizer was the standard rate under both inflorescence removal and non-removal. However, they were higher in inflorescence removal than in non-removal when the amount of fertilizer was increased to twice that of the standard rate.

Productivity of leaves and stems of buckwheat under different fertilization rates and inflorescence removal.

Fresh weight of leaves and stems of sweet buckwheat (Suwon No. 1 & 2) was higher in the inflorescence removal treatment than in inflorescence non-removal. It was higher in Suwon No. 2 than in Suwon No. 1 and was

Table 1. Comparison in photosynthetic rate between inflorescence cutting and non-cutting of buckwheat under the different fertilization.

Cultivar	Evaporation ($mol/m^2/s$)				Stomatal conduction ($mol/m^2/s$)				Assimilation rate ($\mu mol/m^2/s$)			
	Inflorescence cutting		Inflorescence non-cutting		Inflorescence cutting		Inflorescence non-cutting		Inflorescence cutting		Inflorescence non-cutting	
	1X*	2X	1X	2X	1X	2X	1X	2X	1X	2X	1X	2X
Suwon 1	4.94± 0.23	4.54± 0.14	6.53± 1.76	3.52± 0.27	0.17± 0.05	0.12± 0.01	0.28± 0.10	0.10± 0.01	15.23± 0.38	9.11± 0.54	15.00± 1.41	6.75± 1.26
Suwon 2	5.45± 0.58	6.52± 1.22	4.14± 0.64	1.50± 0.24	0.17± 0.02	0.19± 0.05	0.13± 0.02	0.04± 0.01	10.36± 0.14	11.53± 0.79	8.55± 1.10	2.42± 0.38
Clfa 39	4.66± 1.58	5.65± 0.76	6.05± 1.38	7.96± 0.89	0.28± 0.08	0.16± 0.03	0.18± 0.06	0.29± 0.06	16.43± 2.30	9.42± 0.15	10.01± 1.48	12.27± 0.66

1X: Standard fertilization (N-P-K 10-29-20 400 kg/10a)
2X: Increased fertilization (N-P-K 10-29-20 800 kg/10a)

higher under standard fertilization than under increased fertilization.

Among the three cultivars used in this experiment, Clfa 39 had the lowest fresh weight of leaves and stems as shown in Table 2. It is apparent from Table 2 that in both common and Tartary buckwheat, standard fertilization and inflorescence removal resulted in additional fresh weight production.

There is a very important relationship between flower initiation and the amount of blossoming and setting of seed. Ruszkowski (1990) found that the phenomenon of compensation and over compensation between the main stem and branches occurred only when the inflorescences were removed from the main stem. High branching results in most of the nutrients being used for vegetative growth and only small amount are utilized for seed establishment (Kreft, 1986). These informations could be a clue for improving seed productivity of buckwheat. However, there are no previous reports on branch defoliation or removal of inflorescences to improve hay productivity of buckwheat. Bud removal in soybean resulted in an increase in the number of branches but there was no difference in total area and dry weight of the leaves (Hong et al., 1987). The present study showed that the fresh weight of the leaf and stem increased when inflorescences were removed. The number of branches also increased (data not shown). However, the increase of branch number could not be fully responsible to the increase of fresh weight in the leaves and stems. Changes in the amount of carbohydrates and dry matter yield in each organ should be compared between the plants of which inflorescences were normally grown and seeds set after fertilization and the plants of which inflorescences were removed before setting seeds. Halbrecq and Ledent (2001) demonstrated that small limitations of assimilates supplied by the defoliation of the leaves subtending the inflorescences seemed not to be an important factor in the regulation of buckwheat seed setting. An additional study is now in progress to clarify physiologically how inflorescence removal affecta buckwheat hay yield.

Rutin content under the different fertilization and inflorescence defoliation

Table 3 showed that inflorescence defoliation did not increase rutin content in buckwheat regardless of the fertilization level. Rutin content was higher in plants in which the inflorescences were not removed. Furthermore, rutin content decreased markedly under increased fertilization as compared to standard fertilization. The rutin content of buckwheat is known to fluctuate according to environmental conditions such as climate and soil (Shibata et al., 1979; Campbell, 1997). Therefore, single cropping as was done in this study has a limitation for concluding that buckwheat hay may be increased by con-

Table 2. Comparison of fresh weight of leaf and stem between inflorescence cutting and non-cutting of buckwheat under the different fertilization.

Cultivar	Organ	Inflorescence cutting (g)		Inflorescence non-cutting (g)	
		Standard fertilization (1X)	Increased fertilization (2X)	Standard fertilization (1X)	Increased fertilization (2X)
Suwon 1	Leaf	9.97	3.40	3.03	1.13
	Stem	10.40	5.50	3.90	2.73
Suwon 2	Leaf	12.63	4.97	8.23	4.70
	Stem	20.67	7.30	9.87	4.17
Clfa 39	Leaf	6.57	3.80	4.60	3.00
	Stem	7.03	6.10	3.93	3.20

Table 3. Comparison of rutin content in leaf between inflorescence cutting and non-cutting of buckwheat under the different fertilization.

Cultivar	Inflorescence cutting (mg/100 g)		Inflorescence non-cutting (mg/100 g)	
	Standard fertilization (1X)	Increased fertilization (2X)	Standard fertilization (1X)	Increased fertilization (2X)
Suwon 1	57.48	53.83	107.00	60.08
Suwon 2	90.05	34.62	101.48	69.65
Clfa 39	89.85	34.01	112.04	81.20

trolling fertilization and defoliation. Moreover, the effects of fertilization and defoliation on buckwheat hay production would dependent on species, cultivar and the cropping system. Even though inflorescence removal was significantly effective in increasing the amount of buckwheat hay, the practices for flower removal in buckwheat cultivation are very limited in terms of labor and expenses. Therefore, gametocides might be applied to buckwheat inflorescences at the flowering stage as an alternative to produce higher yields of buckwheat hay without considering seed production. Further studies are required on the effect of a gametocides in these buckwheat species.

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Varietal differences of antioxidant activity in Tartary buckwheat flour as evaluated by chemiluminescence

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ABSTRACT

Photon emission from a mixture of Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) flour, H_2O_2 and acetaldehyde was measured as an index of the antioxidant activity of the flour. The photon emission was detected as an image with a CCD -photo camera equipped with a photomultiplier for 10 minutes to determine the intensity of chemiluminescence (CL) from the mixture, and the varietal differences of CL, which represents the antioxidant activity of the flour, were examined using 25 landraces and a standard line that were grown in the same field and at the same time. The relative CL (RCL), the ratio of the CL from 1 g flour to that from 10^{-6} M gallic acid, ranged from 0.311 to 0.950, with the mean and standard deviation of 0.569 and 0.209, respectively. These results suggested that there was a significant genetic difference in the antioxidant activity of the flour among Tartary buckwheat landraces. The landraces collected at a higher altitude in Asia did not show a higher RCL, and the diversity of RCL among the landraces tended to be larger in those collected at a lower altitude than those at a higher altitude.

INTRODUCTION

Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) is a cultivated species related to common buckwheat. Tartary buckwheat adapts to severe environmental conditions in high altitude mountainous areas from Asia to Europe, where other crops do not grow easily. Although Tartary buckwheat had not been well known in Japan in the past, it recently has become popular as a source of healthy food and medicine (Yasuda, 2001; Zhao et al., 2001). One of the reasons for the recently increased interest in Tartary buckwheat is its outstandingly high polyphenol content as compared with common buckwheat and other crops (Minghe and Fukang, 1998). Especially, the rutin content of Tartary buckwheat flour which is approximately nine times higher than that of common buckwheat (Wang et al., 1995). Although foods containing such compounds usually taste bitter, Tartary buckwheat is accepted by consumers because they appreciate its important functional substances for human health in an aging society.

The genetic resources of Tartary buckwheat which have been collected so far are relatively abundant, but their usefulness as resources for a breeding program has not been well evaluated. Therefore, our laboratory has been studying the varietal difference of the characteristics related to productivity and food quality of Tartary buckwheat landraces collected from around the world. Geographical variations in morphological characteristics (Yoshida et al., 1995, 1997), seed storage protein and DNA (Taniguchi, 1996) and amylose content in the flour (Inoue and Tanaka, 2002) have been reported. However, there are few reports on the chemical composition of Tartary buckwheat flour in relation to its function as a food stuff. Limited information is available on the genetic variation of the characteristics of Tartary buckwheat flour in relation to its function as healthy food and the substances which are related to this function. Studies on the varietal difference and the geographical distribution of antioxidant activity may contribute to finding the hot spot with a higher genetic diversity and can help promote developments in breeding programs.

Since Tartary buckwheat is distributed at a higher altitude than other crops, it is considered that the species has been exposed to and evolved under strong stresses of light, temperature, wind and water. Plants generate a large amount of active oxygen under such stress conditions. This phenomenon is commonly observed and has been named as an oxidative burst (Doke, 1996). Plant pigments are known to be produced under such oxidative conditions. Asada (1999) pointed out that the ingenious antioxidant mechanism was constructed in chloroplasts. Thus, Tartary buckwheat is considered to have a high radical scavenging or antioxidant activity to avoid injury by active oxygen. From the viewpoint of functional food

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Abbreviations: Amount of emitted photons (P), Chemiluminescence (CL), Gallic acid (GA), Hydrogen donor (Y), Hydrogen peroxide (H_2O_2), Mediator (Z), Oxygen species (X), Relative CL (RCL)

science, landraces which have long been subjected to stress conditions during their history of evolution from wild plants should be good resources for the development of new healthy foods with high antioxidant activity. Varietal differences of antioxidant activity in common buckwheat were reported by Morishita et al. (2002) and Watanabe et al. (1995), but there have been no reports on Tartary buckwheat. In an attempt to develop new Tartary buckwheat varieties with high functionality as a food, we analyzed the antioxidant activity of flour, and examined its varietal differences and geographical distribution.

MATERIALS AND METHODS

Plant materials

Twenty five landraces of Tartary buckwheat, collected and stored at Shinshu and Kyoto Universities, 13 Nepalese, nine Chinese, two Bhutanese and one Slovenian landrace, and an additional Japanese line (Table 1) were used. The Japanese line, Hokuriku No. 4, which was recently developed by Hokuriku Research Center, National Agricultural Research Center in Japan, was used as a standard to compare with the landraces. The landraces were chosen as those having a similar maturation date, high lodging resistance and high yielding ability based on the results of a field culture trial in 2000. The collection sites of the landraces were distributed at altitudes from 700 m in Nepal to 3880 m in Tibet, and at an latitude from 46 N in Slovenia to 23 N in the southern part of Yunnan Province in China.

The landraces and the line were grown in a field of Chushin Agricultural Experiment Station, Nagano Prefecture (latitude 36°N, 740 m above sea level). Sowing time was at 14th July and harvest time was at 19th, 24th,

Table 1. Plant materials and the data of collection site.

ID No.	collection site			Altitude (m)	Latitude
	Accsession No.	Village (Town)	Country		
1	C92-26	Shigatse	China	3880	29 12'
2	B9126	Longtola	Bhutan	3070	27 30'
3	IR-85-9131-C	Thinger	Nepal	3700	29 00'
4	Col# 36	Jharkot	Nepal	3400	28 48'
5	Col# 45	Chhenga	Nepal	3400	28 48'
6	N 8629	Chahishe	Nepal	700	27 30'
7	N 8630	Chakulu	Nepal	1300	27 30'
8	C 9048	Simao	China	1100	23 00'
9	IR-85-6378-B	Badhu	Nepal	1140	29 24'
10	IR-85-6224-B	Makai	Nepal	1360	29 48'
11	C 92-18	Longxi	China	1620	35 00'
12	C 92-19	Longxi	China	1620	35 00'
13	C 9042 (black)	Hequing	China	—	26 30'
14	MY-2-6	Gomanek	Nepal	2250	28 48'
15	N8629	Chalise	Nepal	—	27 30'
16	B 9125	Nobding	Bhutan	2530	27 30'
17	C 9126	Tianba	China	—	26 30'
18	C 9042 (white)	Hequing	China	—	26 30'
19	U-8004 (S)	Jumla	Nepal	2350	29 18'
20	IR-85-7-B	Sarkeghat	Nepal	1805	30 00'
21	C 9039	Zhaojiao	China	—	28 00'
22	C 05 60A	Afi	China	—	41 00'
23	IR-85-9135-B	Thinger	Nepal	3700	29 00'
24	#233	Rodohova Vas	Slovenia	—	46 00'
25	IR-85-9009-B	Sammar	Nepal	2800	29 00'
26	Hokuriku No. 4*		Japan	—	—

*: Standard line

26th October in 2000. The experiment was conducted by randomized block design. Planting density was 40 plants/m^2. The amount of fertilizer which was applied was as follows; N; 0.25 kg/a, P_2O_5; 0.25 kg/a, K_2O; 0.20 kg/a and dolomite; 10 kg/a, respectively. The field soil type was Andosol.

Fully ripened seeds were harvested at approximately 100 days after seeding, and stored at 5°C in a cooling box. After removing hull by using a food-processing mill, FM-33 (San Co. Ltd., China), all of the groat was milled with a high-speed centrifugal mill, P-14 (FRITSCH JAPAN Co., Ltd., Yokohama, Japan) and passed through a 0.5 mm mesh to increase particles uniformity.

Measurement of chemiluminescence

The chemiluminescence (CL) intensity was measured by using an XYZ system (Yoshiki et al., 1995a). Low-level chemiluminescence is emitted from hydrogen peroxide (H_2O_2) solution mixed with phenols and aldehyde (Trautz und Schorigin, 1905). Yoshiki et al. (1995a, b, 1998) developed a convenient measuring system (XYZ system) for evaluating the antioxidant activity of crops and foods using this principle.

The XYZ system proposed by Yoshiki et al. (1998) consists of three chemical compounds: reactive oxygen species (X), hydrogen donor (Y) and mediator (Z). In this XYZ system, the amount of emitted photons (P) is highly correlated with the concentration of X, Y and Z species, and is shown by the equation, $P=k[X]\cdot[Y]\cdot[Z]$, where k is a constant. They concluded that the photon emission from the XYZ system was attributable to the energy transition that occurred when X was scavenged by Y and Z. Thus, if the sample material is used as Y, its reactive oxygen-scavenging activity is evaluated by the amount of emitted photons. It was also shown that the amount of photon emitted from the system coincides well with the results obtained by HPLC-electrochemical methods and the DPPH scavenging activity (Akiyama et al., 2001).

Hydrogen peroxide (H_2O_2) at 196 mmol/L was used as a reactive oxygen species, and 360 mmol/l of acetaldehyde saturated with potassium hydrogen carbonate as a mediator. The hydrogen peroxide (H_2O_2) and acetaldehyde were products of Kanto Chemical Co., Inc. Japan. Potassium hydrogen carbonate and gallic acid (GA; 3,4,5-trihydroxybenzoic acid) used as the standards were products of Nacalai Tesque, Inc. Japan and Wako Pure Chemicals Industries, Ltd. Japan, respectively.

A flour sample (0.2 g) was put into each well (inside diameter, 15 mm) on a 12-well plate. Four wells on a well plate were used per landrace or line for replication. The flour samples were soaked with 500 μl distilled water in each well and were kept at 25°C for 20 minutes to decrease the variance due to pipetting, then 300 μl each of H_2O_2 and acetaldehyde solutions were injected into each well. The wavelengths of the peaks of photon emission spectra from GA and Tartary buckwheat flour samples in this XYZ system were 630 nm in the spectrophotometric analysis with a CLA-SP2 (Tohoku Electric Engineering, Sendai, Japan) conducted prior to this experiment. Photon emission at about 350–750 nm was detected and recorded as a 2-D image by using a CCD-photo camera equipped with a photomultiplier, Aquacosmos/VIM (Hamamatsu Photonics Co. Ltd., Hamamatsu, Japan) for 10 minutes at 25°C starting immediately after injecting the H_2O_2 and acetaldehyde solutions. Image processing was applied to the recorded image to quantify photon emission, and CL was determined on the basis of the number of photons emitted from 1 g of flour. The relative CL (RCL) was calculated as the CL at 10^{-6} M GA, according to the relationship between concentration of GA and CL (Fig. 1).

Analysis of variance was applied to clarify the varietal differences in CL and RCL.

RESULTS

Detection of photon emission was started immediately after adding H_2O_2 and acetaldehyde solutions to the flour samples, because the response of photon emission takes place immediately. The photon emission started quickly during the first five minutes and was relatively slow during the next five minutes. Fig. 2 shows an example of photon emission from a Tartary buckwheat flour sample placed on a well plate. The number of photons emitted is indicated by a color in the color scale shown to the right of the image. White and blue correspond to the maximum and minimum values, respectively.

Chemiluminescence (CL) and RCL values of each lan-

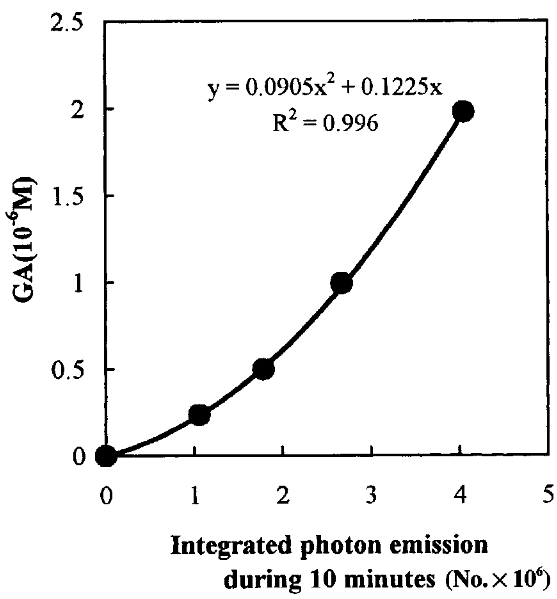

Fig. 1. Relationship between CL and gallic acid (GA) concentration.

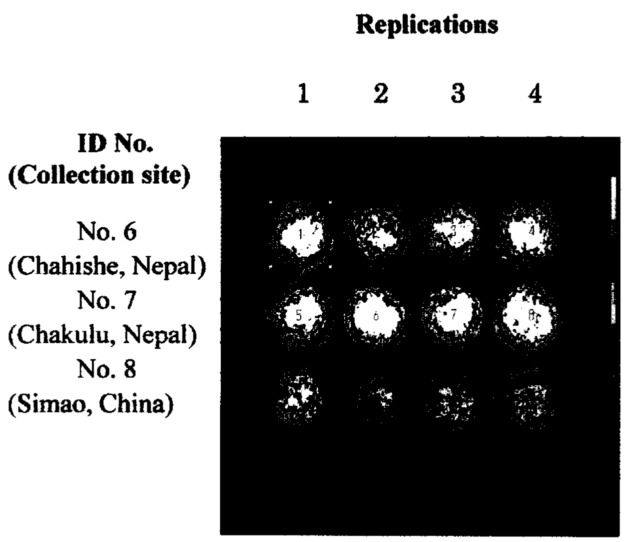

Fig. 2. Integrated image of photon emission on a micro well plate (3×4 wells).

drace are shown in Table 2. RCL values varied from 0.950 to 0.311 with the mean and standard deviation 0.569 and 0.209, respectively. No correlation was found between the geographical position where the landraces were collected and CL or RCL. The highest RCL was observed in a Nepalese landrace, and the lowest RCL in a Slovenian landrace. The highest landrace had a 1.85 times larger RCL than the standard line, Hokuriku No. 4. The results of analysis of variance are shown in Table 3. There was a significant difference among the Tartary buckwheat landraces examined.

Since the collection sites of the landraces used in this experiment was dispersed widely in Asia, geographical characteristics of their collection sites greatly varied as shown in Table 1. The relationship between RCL and latitude or altitude of a collection site was analyzed to clarify the influence of meteorological environment of the collection site on the genetic variation in antioxidant

Table 2. Antioxidant activity of Tartary buckwheat flour.

ID No.	Accession No.	CL from 1 g flour of Tartary buckwheat	RCL (Relative to CL from 10^{-6} GA)	Ratio of RCL to that of comparative variety (ID No. 26)
7	N 8630	$500\times10^4\pm600\times10^3$	0.950	1.854
14	MY-2-6	$499\times10^4\pm119\times10^3$	0.948	1.851
8	C 9048	$499\times10^4\pm616\times10^3$	0.948	1.851
15	N8629	$489\times10^4\pm983\times10^3$	0.919	1.794
21	C 9039	$446\times10^4\pm396\times10^3$	0.805	1.572
6	N 8629	$423\times10^4\pm400\times10^3$	0.744	1.452
13	C 9042	$402\times10^4\pm866\times10^3$	0.692	1.351
10	IR-85-6224-B	$394\times10^4\pm915\times10^3$	0.673	1.313
16	B 9125	$373\times10^4\pm467\times10^3$	0.624	1.218
19	U-8004 (S)	$370\times10^4\pm164\times10^3$	0.616	1.202
18	C 9042	$335\times10^4\pm446\times10^3$	0.538	1.050
17	C 9126	$333\times10^4\pm419\times10^3$	0.532	1.039
12	C 92-19	$331\times10^4\pm864\times10^3$	0.528	1.031
11	C 92-18	$329\times10^4\pm853\times10^3$	0.524	1.023
26	Hokuriku No. 4*	$324\times10^4\pm610\times10^3$	0.512	1.000
1	C92-26	$303\times10^4\pm251\times10^3$	0.468	0.914
2	B9126	$302\times10^4\pm221\times10^3$	0.466	0.909
9	IR-85-6378-B	$292\times10^4\pm757\times10^3$	0.446	0.871
4	Col# 36	$282\times10^4\pm433\times10^3$	0.424	0.829
23	IR-85-9135-B	$263\times10^4\pm844\times10^3$	0.388	0.757
22	C 05 60A	$259\times10^4\pm376\times10^3$	0.380	0.742
5	Col# 45	$247\times10^4\pm541\times10^3$	0.357	0.698
25	IR-85-9009-B	$235\times10^4\pm707\times10^3$	0.335	0.653
3	IR-85-9131-C	$233\times10^4\pm396\times10^3$	0.330	0.645
20	IR-85-7-B	$230\times10^4\pm460\times10^3$	0.326	0.637
24	#233	$222\times10^4\pm808\times10^3$	0.311	0.606
Mean		343×10^4	0.569	—
Standard deviation		90×10^4	0.209	—

Table 3. Analysis of variance for CL in Tartary buckwheat flour.

Factor	d.f.	SS	MS	F	Probability
Total	131	6.029×10^{12}			
Landraces	25	3.982×10^{12}	1.593×10^{12}	8.249	2.265×10^{-15}
Error	106	2.047×10^{12}	1.931×10^{10}		

activity. Although no significant correlation was found between RCL and latitude, landraces collected at a higher latitude tended to have lower RCL. A similar relationship was found between RCL and altitude (Fig. 3). A high RCL was not observed in landraces collected at a high altitude, and the diversity of RCL was relatively larger in landraces collected at a lower altitude than in those collected at a higher altitude.

The landraces that had a high RCL were those collected at a lower altitude located in the warm mountainous areas around the Tharai Basin in Nepal and the southern part of Yunnan Province in China.

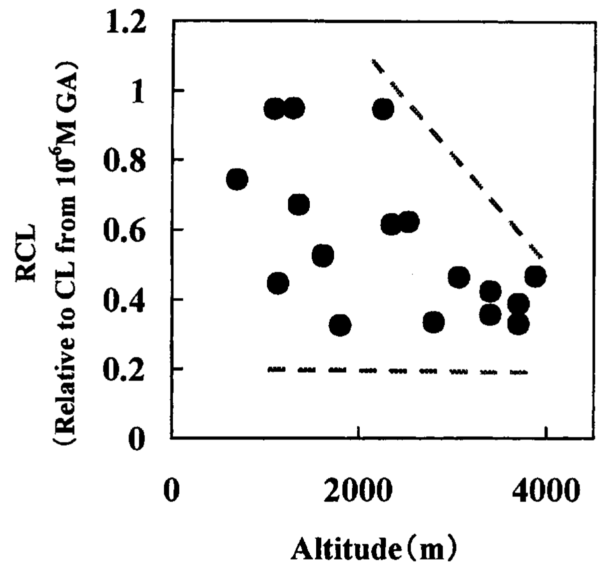

Fig. 3. Relation between antioxidant activity and altitude of collection site.

DISCUSSION

Measurement of CL by the XYZ system has been reported as a convenient method to evaluate the antioxidant activity of foods and beans (Yoshiki et al., 1995a, b; Yoshiki and Okubo, 1998) and cereals (Akiyama et al., 2001). Since the photon emission from Tartary buckwheat flour in the XYZ system started quickly in the early stage of measurement (the first five minutes after the injection of H_2O_2 and acetaldehyde solutions into a sample flour), the compounds in the flour that responded quickly to active oxidants are considered to be related to the varietal differences for antioxidant activity.

Landraces used in this experiment were all medium maturty, and thus there was little difference in the dates of flowering and maturation. It is safe to say therefore that the varietal difference in the antioxidant activity of flour revealed in this study was caused by genetic factors and not by other factors such as environmental conditions during the grain-filling stage.

Yoshida et al. (1995, 1997) and Inoue and Tanaka (2001) examined the geographical variation of Tartary buckwheat in agro-ecological characteristics and amylose contents of flour. They suggested that there were two different genetic groups in the Nepalese mountainous region. Taniguchi (1996) investigated the geographical distribution of genotypes of Tartary buckwheat based on RAPD analysis and the analysis of the seed storage proteins, and recognized the distribution of three genetic groups in Nepal. The results suggested that there was a genetic diversity in Tartary buckwheat from the Himalayan region in Nepal. Furthermore, the difference in agricultural systems is also striking between the high mountainous zone at an altitude of 4000 m and the Indian plain which is below an altitude of 1000 m (Yoshida et al., 1997). The antioxidant activity seems to coincide with the agro-ecological characteristics of the geographical distribution. Thus, the warm areas in Himalayas and southern China are assumed to be a hot spot of intraspecific diversity of the antioxidant activity in Tartary buckwheat flour.

In the present experiment, none of the landraces collected from a high altitude gave a high value of RCL. Tartary buckwheat was assumed to have originated from somewhere in the mountainous region in the western part of Sichuan or from the northwestern part of Yunnan in China (Tuji and Ohnishi, 2000). Nepalese and Chinese landraces which are distributed in the low altitude areas were derived from the landraces distributed at a high land altitude area mentioned above. Although the reason why the landraces that were collected at a lower altitude widely varied in antioxidant activity (Fig. 3) was not clear, however, we presumed that they adapted to light and temperature conditions during their diffusion processes.

Higher plants commonly have the metabolism to protect against oxidative stress, and flavonoid substances are assumed to play an important role in the protection against peroxidation of squalene induced by UV-B irradiation in crop grains (Osawa, 1994). In Tartary buckwheat, seed viability after a one-year storage is higher than that in common buckwheat (unpublished). This species might have acquired a tolerance to UV-induced oxidative stress at a higher altitude.

Arakawa et al. (1986) reported a varietal difference in the rate of anthocyanin synthesis in apple under UV-B irradiation. Flavonoids and phenolic acids in apple are highly influenced by UV-B and temperature (Lancaster et al., 2000), but the effects of UV-B irradiation and temperature vary with the cultivar. In *Polygonum*, the anthocyanin accumulation varied with the temperature during UV-B irradiation (Yamaguchi et al., 2000). Luthar and Kreft (1999) pointed out that tannin content was influenced by the temperature during the grain-filling stage in common buckwheat. These reports suggest that the production of substances related to antioxidant activity varies with the genotype, light quality and temperature. In other words, the antioxidant activity is determined by the genotype× environment interaction in many plant species.

Since the seeds used in this experiment were harvested from a field located at an altitude of 740 m, the temperature and the intensity of UV irradiation in the field differed from that at the site of origin of the landraces and most of the seed collection sites. The effect of temperature and light on the antioxidant activity of flour requires analysis in the future.

It is commonly recognized that landraces are adapted to the local meteorological environment of the specific area where they are growing. Watanabe et al. (1997) pointed out that antioxidant compounds contained in common buckwheat hulls function as a bactericide, fungicide and viricide in plant tissue. We presume that a species which spread from the site of origin to a region of warm and humid meteorological condition, might not have only antioxidant activity but could also have chemical defense activities against bacteria, fungi and viruses.

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Fagopyrum 20: 53–58 (2003)

Comparison of Mechanical and Chemical Characteristics between Common and Tartary Buckwheat

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ABSTRACT

The mechanical and chemical characteristics of buckwheat flour and dough were compared between common and Tartary buckwheat. Mechanical analysis showed a striking difference in mechanical characteristics between the two species, and chemical analysis demonstrated that the protein composition of the flour is also different. A marked difference was found in color, i.e. in a^* and E values between common and Tartary buckwheat dough. The implications of the present findings are discussed from the viewpoint of food processing.

INTRODUCTION

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

A major difference between the two different cultivated species, i.e., common buckwheat (*F. esculentum* Moench) and Tartary buckwheat (*F. tataricum* Gaertner), is that common buckwheat is utilized worldwide, whereas Tartary buckwheat is utilized as a traditional food in relatively limited regions such as in southern China (Zhang et al., 2003), Bhutan (Norbu and Roder, 2003), and the Himalayan hills from northern Pakistan to eastern Tibet (Ohnishi, 2003). Recent studies have suggested that Tartary buckwheat may exhibit beneficial effects on human health (e.g. Lin et al., 1998), but the exact mechanisms involved in the beneficial effects remain uncertain. Thus a large amount of attention has been currently paid to Tartary buckwheat (Katayama, 2001) and the development of new products made from Tartary buckwheat is currently the subject of great interest. Despite of importance of Tartary buckwheat as a food, the scientific food characterization of Tartary buckwheat remains to be fully examined. Characterization of Tartary buckwheat is an important subject from the view of food processing.

The present study was conducted to compare the mechanical characteristics of flour and dough between Tartary buckwheat and common buckwheat.

MATERIALS AND METHODS

Materials

Six buckwheat samples were analyzed in this study. They were two varieties of common buckwheat, i.e., var. Kitawase-soba (abbreviated as CB-1) and var. Hashikami-wase (CB-2), and four varieties of Tartary buckwheat. The Tartary buckwheat samples consisted of one Japanese sample and three Chinese samples. The Japanese sample, i.e., var. Hokkei No. 1 (TB-JP) was obtained from the Department of Crop Breeding, Hokkaido National Agriculture Experiment Station (Hokkaido, Japan). Three Chinese Tartary buckwheat samples were supplied by Prof. Rufa Lin; They were var. Hei Ku Qiao (TB-CN-1), var. Hui Ku Qiao (TB-CN-2) and var. Shoungang Bendi Ku Qiao (TB-CN-3). The last one was provided as flour. Buckwheat grain of each sample were milled using a roller mill (Quadrumat Junior, Model No. 279002, Brabender OHG Duisburg, Germany) fitted with 231 μm sieve. The buckwheat flour thus obtained was subjected to analysis.

Mechanical measurements

Mechanical characteristics of common and Tartary buckwheat were evaluated using five different mechanical analyses (Kawabata, 1989): texture analysis, creep analysis, stress relaxation analysis, breaking analysis and tensile analysis. Texture analysis of the buckwheat dough was performed with a Rheolometer RX-1600 (Iio Denki Co., Japan) according to the procedure previously described by Ikeda et al. (1997, 1999). Creep analysis for six mechanical factors with heated buckwheat dough was

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performed with a Rheoner RE -3305 (Yamaden Co. Ltd., Tokyo, Japan) using a previously described procedure (Ikeda and Asami, 2000; Ikeda et al., 2001). Stress relaxation analysis with two mechanical factors with heated buckwheat dough was performed using a Rheometer RT-3005D (Rheotec Co., Ltd., Tokyo) using a previously described procedure (Ikeda and Asami, 2000). Breaking analysis with buckwheat noodles was performed with a Rheometer RT-3005D using the procedure of Ikeda and Asami (2000). Tensile analysis of buckwheat noodles was performed with a Rheoner RT-3005D using a previously described procedure (Ikeda and Asami, 2000). Mechanical measurements of heated buckwheat dough samples were repeated five times, each time with a different sample of buckwheat dough. Breaking characteristics, i.e., breaking stress and rupture energy, of buckwheat dough were analyzed with a Rheoner RE-3305 as previously described by Ikeda and Asami (2000).

Extraction and electrophoresis

For chemical analysis and electrophoresis of the buckwheat proteins in the cooked dough samples, which were subjected to mechanical measurements, the dough samples were first lyophilized and then ground into flour with a mixer B-400 (Büchi Co, Switzerland) and a mortar. The combined fraction of buckwheat albumin plus globulin was extracted using the procedure of Ikeda and Asami (2000). Stated briefly, the flours, which were obtained above, were extracted with ten-fold (v/w) volume of 0.1 M Tris-HCl buffer (pH 8.0) for 1 hr at 4°C. After extraction, the suspensions were centrifuged at 17,000 g for 20 min, and then 10 μl of the supernatants was subjected as the combined fraction of buckwheat albumin plus globulin to polyacrylamide gel electrophoresis with sodium dodecylsulfate (SDS-PAGE). The supernatants were also subjected to a protein assay using the method of Lowry et al. (1951). 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 a 7% acetic acid solution. Bovine serum albumin (66 kDa), trypsinogen (24 kDa), β-lactoalbumin (18.9 kDa), and lysozyme (14.3 kDa) were used as molecular weight markers. Electrophoregrams were screened to identify protein bands and the concentration of these protein bands was determined by a Pharmacia PDI-Imaging Analyzer (Amersharm Pharmacia Biotech., Sweden). The combined fraction of buckwheat glutelin plus prolamin from the flour samples was examined using the procedure of Ikeda and Asami (2000).

Analysis of protein

The total crude protein content ($N\times 6.25$) of the buckwheat flours was determined using a Kjeltec Auto 1035 nitrogen analyzer (Perstorp Analytical Tecator, Sweden). Protein assay in solution was determined using the method of Lowry et al. (1951).

Measurement of color

Color (L^*, a^*, b^*, C^* and ΔE) of buckwheat samples was measured using a Color Reader CR-13 (Minolta, Tokyo, Japan). L^* indicates lightness in color (CIE, 1976). The values of a^* and b^* indicate the combined value of hue and chroma; a^*, color direction from red to green, and b^*, color direction from yellow to blue (CIE, 1976). The C^* value, which is estimated by $\{(a^*)^2+(b^*)^2\}^{1/2}$, indicates the total chroma (CIE, 1976). The ΔE value, the magnitude of the total color difference, which is widely utilized in food products including cereal products (Good, 2002), is estimated by $\{(\Delta a^*)^2+(\Delta b^*)^2+(\Delta c^*)^2\}^{1/2}$. The ΔE value between the common and Tartary buckwheats was estimated by subtracting the averaged E value of common buckwheat from that of Tartary buckwheat.

Statistical analysis

Statistical analysis of correlation and Student's t-test were performed using a personal computer with the programs Stat-View (SAS Institute Inc.) and Excel (Microsoft Co., USA).

RESULTS AND DISCUSSION

Mechanical characteristics of common and Tartary buckwheat

Fig. 1 shows the textural characteristics of the dough from common and Tartary buckwheat. There was a difference in textural characteristics between common and Tartary buckwheat. Greater hardness and lower cohesiveness were found in Tartary buckwheat. In fact, the hardness to cohesiveness ratio of common buckwheat was 1.00, whereas this ratio was 1.70 for Tartary buckwheat.

Fig. 2 shows the creep characteristics with six mechanical elements of common and Tartary buckwheat. Higher elasticity values in E0, E1 and E2 and higher viscosity values in η_1, η_2 and η_N, characterized Tartary buckwheat (Fig. 2). Stress relaxation analysis with two mechanical factors showed higher elasticity and viscosity in Tartary buckwheat as compared with common buckwheat (data not shown).

Fig. 3 shows breaking characteristics of noodles made from common and Tartary buckwheat. Higher rupture energy characterized Tartary buckwheat. Tensile analysis with noodles showed higher tensile strength in Tartary buckwheat, whereas low maximum elongation was found in Tartary buckwheat (Fig. 4).

Fig. 5 shows cluster analysis of two common and four Tartary buckwheat samples with respect to the observed mechanical values (Figs. 1, 2, 3 and 4, and stress relax-

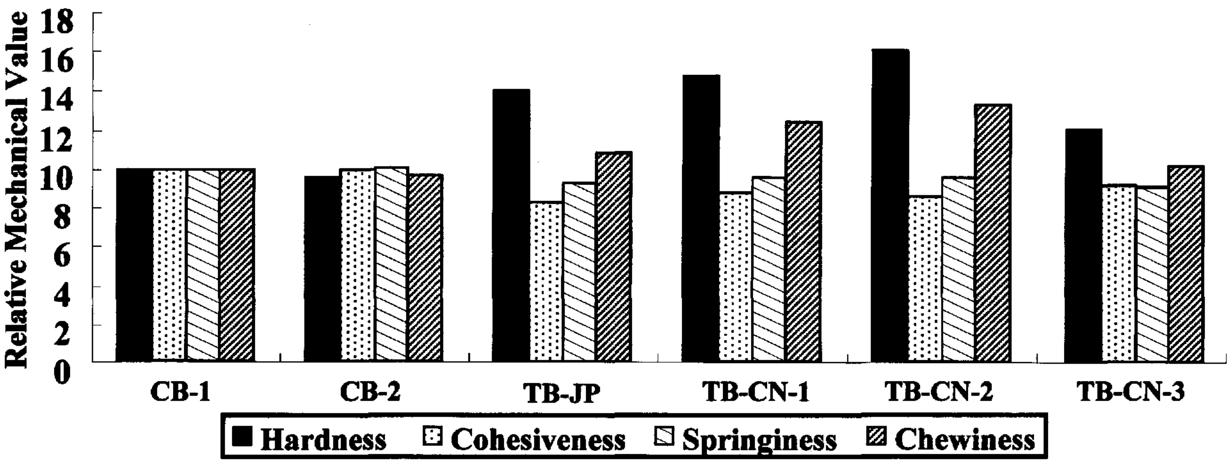

Fig. 1. Textural characteristics of common and Tartary buckwheat doughs. Each analytical values was expressed as a relative textural value as the observed textural value of a common buckwheat (CB-1, i.e., var. Kitawase-soba) as a standard value was taken as 10.00. The abbreviations for buckwheat samples were given in Materials and Methods.

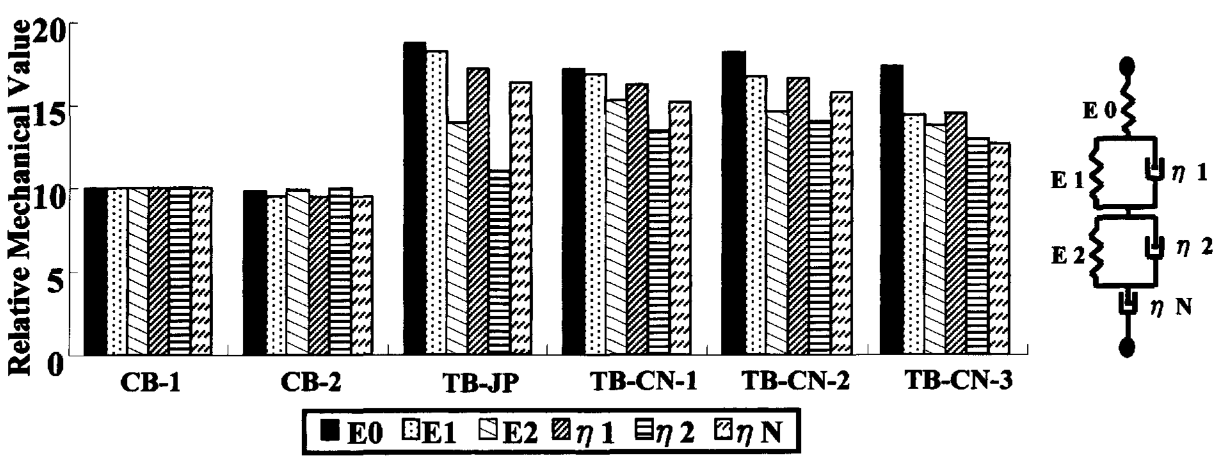

Fig. 2. Creep characteristics of common and Tartary buckwheat doughs. The abbreviations for the buckwheat samples were the same in the Fig. 1.

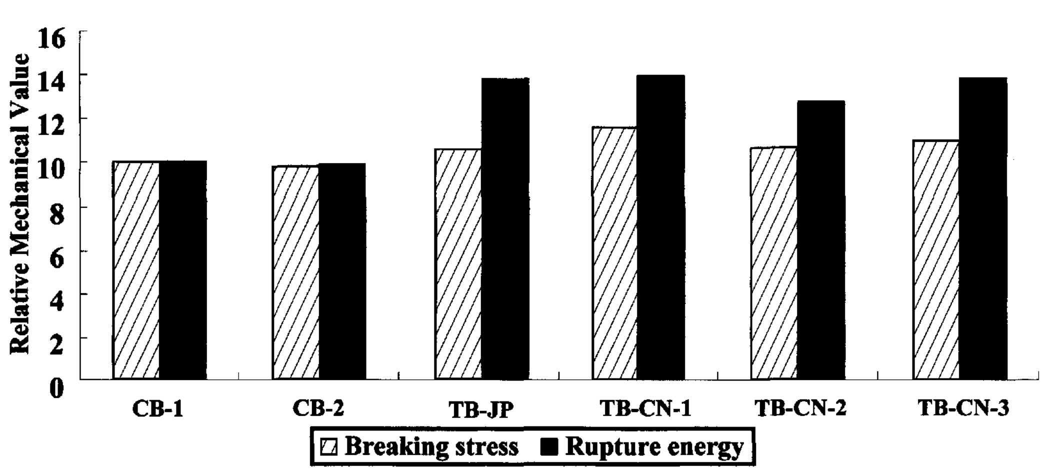

Fig. 3. Breaking characteristics of noodles made from common and Tartary buckwheats. The abbreviations for the buckwheat samples were the same in the Fig. 1.

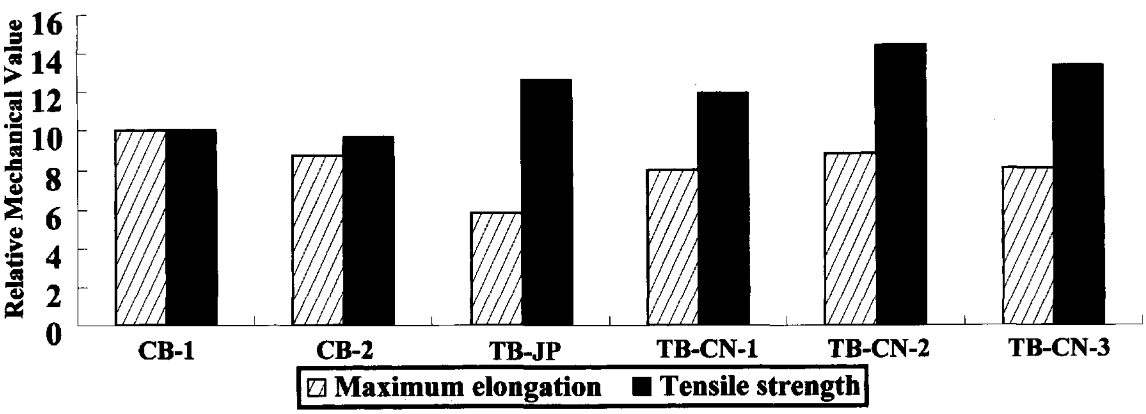

Fig. 4. Tensile characteristics of noodles made from common and Tartary buckwheats. The abbreviations for the buckwheat samples were the same in the Fig. 1.

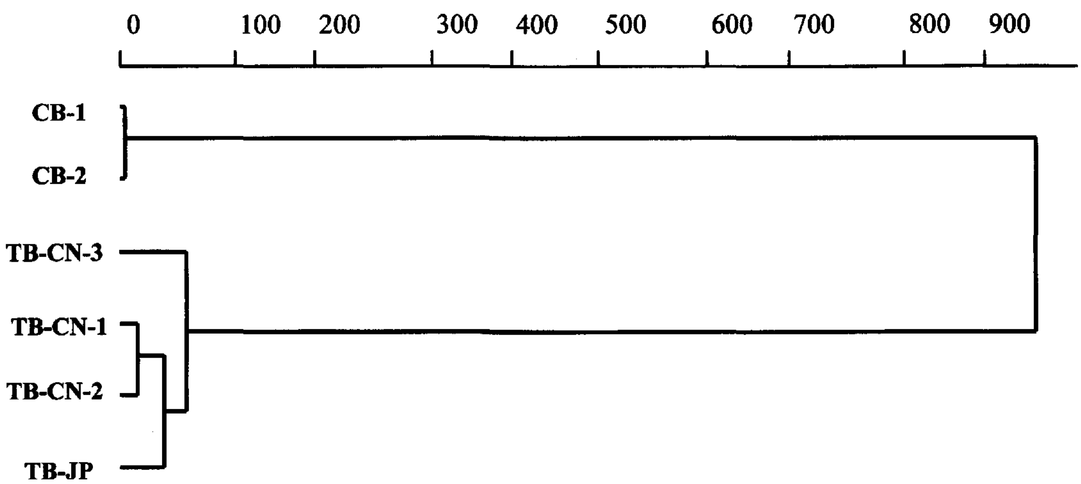

Fig. 5. Cluster analysis of two common buckwheat and four Tartary buckwheat with respect to the observed Mechanical characteristics (Figs. 1, 3, 4, 5 and 6).

ation analysis (data not shown)). Interestingly, cluster analysis (Fig. 5) showed a string difference in the mechanical characteristics between common and Tartary buckwheat.

Color of common and Tartary buckwheat

Fig. 6 shows the color characteristics, i.e., L^*, a^*, b^* and C^*, of common and Tartary buckwheat dough. Fig. 6 also shows the E value between common and Tartary buckwheat. A remarkably high a^* value was found in Tartary buckwheat dough (Fig. 6). ΔE was about 7.3-fold higher in Tartary buckwheat than in common buckwheat. On the other hand, Tartary buckwheat dough showed a lower L^* value as compared with common buckwheat dough (Fig. 6). There was no substantial difference in the b^* and C^* values between common and Tartary buckwheat dough (Fig. 6).

A striking difference in the ΔE value between common and Tartary buckwheat dough was found (Fig. 6). About a 42-fold higher ΔE value was found in Tartary buckwheat. Analysis using L^*, a^*, b^*, C^* and ΔE was sufficient to characterize the color of products made from common or Tartary buckwheat.

Protein compositions of common and Tartary buckwheat

Fig. 7 shows the content of total protein, the combined fraction of albumin plus globulin, and the combined fraction of glutelin plus prolamin in common and Tartary buckwheat flour. A significantly ($P<0.05$) higher content of the glutelin plus prolamin fraction was found in Tartary buckwheat flour than was found in common buckwheat flour.

Fig. 8 shows the SDS-PAGE patterns of the combined fraction of albumin plus globulin and the combined fraction of glutelin plus prolamin. SDS-PAGE analysis showed that there was a striking difference in both the combined fraction of albulin plus globulin and the combined fraction of glutelin plus prolamin between common buckwheat and Tartary buckwheat flour. In addition, as

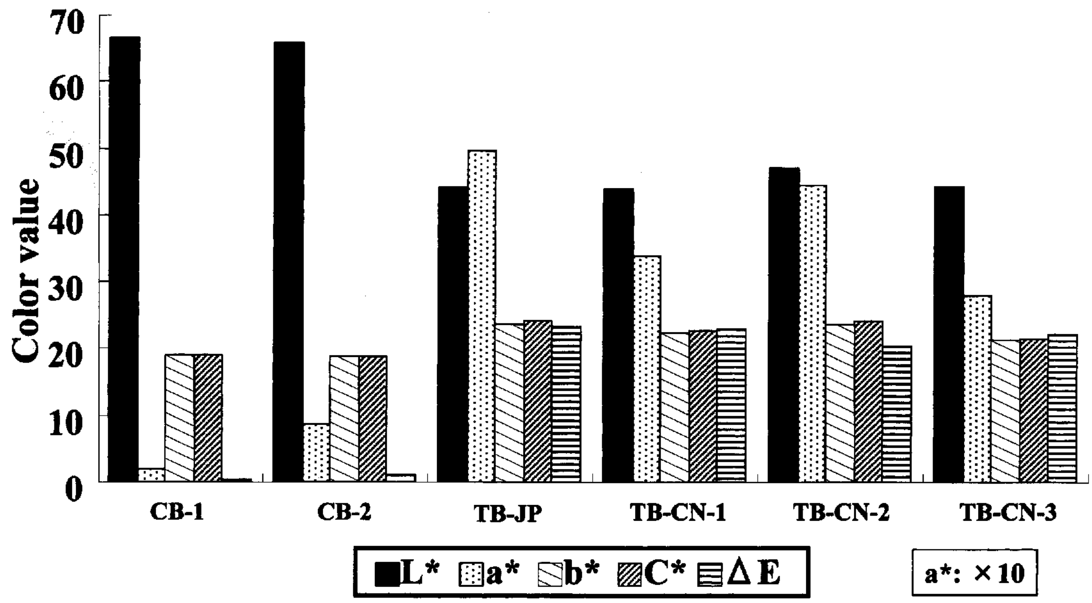

Fig. 6. Color characteristics (L^*, a^* and b^*, C^* and ΔE) of common and Tartary buckwheats. The abbreviations for the buckwheat samples were the same in the Fig. 1.

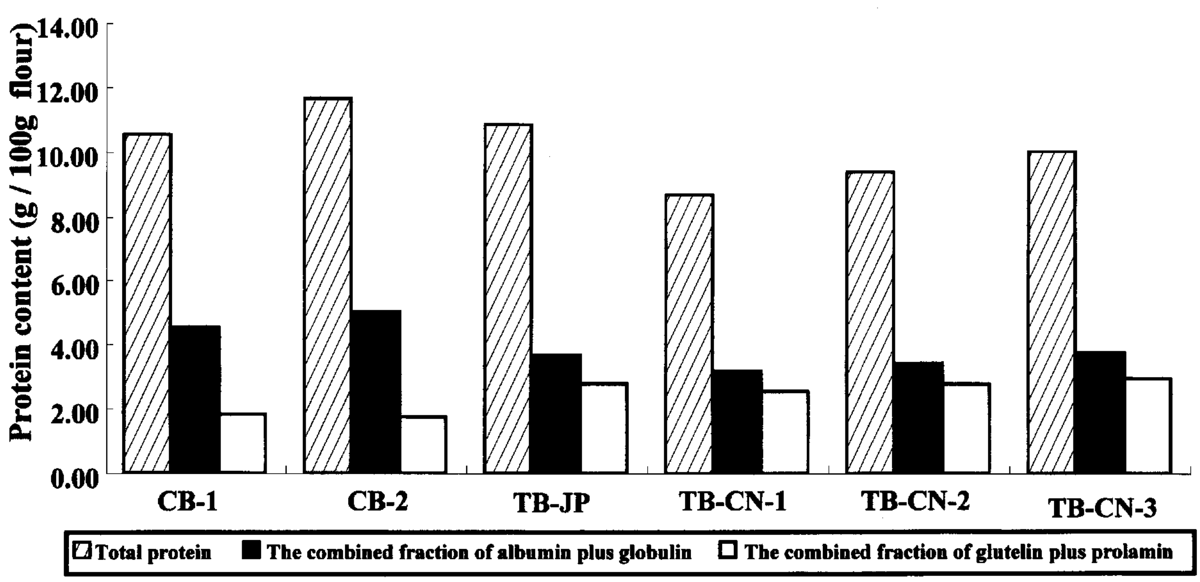

Fig. 7. Contents of total protein, the combined fraction of albumin plus globulin, the combined fraction of glutelin plus prolamin combined fraction of common and Tartary buckwheats. The abbreviations for the buckwheat samples were the same in the Fig. 1.

indicated by the arrows in Fig. 8, there was a difference in protein composition between the Japanese Tartary buckwheat variety and the Chinse varieties.

The observed difference in protein composition between common and Tartary buckwheat may be associated with the observed difference in the mechanical characteristics (Figs. 1 to 5) between common and Tartary buckwheat, but the detailed mechanisms involved remains uncertain. In wheat, the relationship of wheat protein to its functionality has attracted the attention of many cereal chemists (e.g. Macritchie, 1992). Clarifying the relationship of the protein compositions to the mechanical characteristics between common and Tartary buckwheat, will be a subject of great interest in near future.

Buckwheat (*Fagopyrum* spp.) including both common and Tartary buckwheat is widely utilized in the world. Interest in buckwheat for human health, especially Tartary buckwheat is growing rapidly (Katayama, 2001). In the background of such interest, the increasing attention is currently paid to the development of new processed products developed from Tartary buckwheat. This paper has presented some scientific food characteristics of Tartary

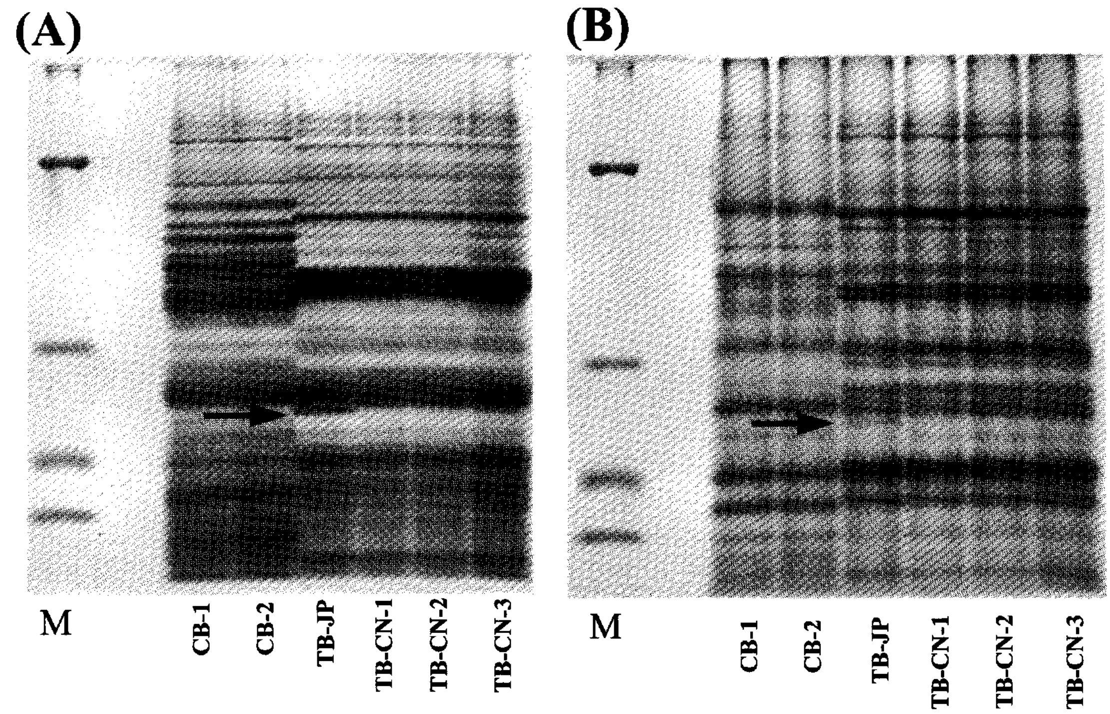

Fig. 8. SDS-PAGE of total protein, the combined fraction of albumin plus globulin, the combined fraction of glutelin plus prolamin of common and Tartary buckwheats. The abbreviations for the buckwheat samples were the same in the Fig. 1.

buckwheat as compared to common buckwheat. The present findings will hopefully stimulate further development to the direction of new products from Tartary buckwheat.

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Correlation between mechanical characteristics of buckwheat dough and constituent properties of buckwheat flour from 12 varieties

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ABSTRACT

Dough properties (toughness and hardness of dough and hardness of boiled dough) and flour properties (gelatinization property (RVA), flour-swelling power, starch-swelling power, crude protein content, water-soluble protein content and crude fat content) were measured in 12 buckwheat varieties and the correlation between these characteristics were investigated. The toughness and hardness of dough had a relationship to the water-soluble protein content. The dough became softer and less fragile when the flour contained more water-soluble protein. The water-soluble protein content had a significant correlation ($r=0.819^{**}$) with the crude protein content and could be measured with a relatively small amount of flour. The hardness of the boiled dough had a significant relationship to starch properties such as RVA peak viscosity, RVA final viscosity, starch-swelling power and flour-swelling power. The boiled dough became softer when the RVA peak and final viscosity were higher and the swelling power of starch and flour were larger. Since the flour-swelling power could be measured using a smaller amount of flour and a shorter analysis time than RVA, it may be useful in the selection in early generations of buckwheat breeding programs. Moreover, starch-swelling power might be a heritable characteristic since varietal differences in starch-swelling power were conserved between the products in 2001 and 2002.

INTRODUCTION

Although buckwheat is processed into various food forms in the world, it is mainly consumed as noodles in Japan. Making buckwheat noodles is more difficult than making wheat noodles as buckwheat flour does not contain gluten. The fragileness of buckwheat dough is a defect in its handling and the improvement of this mechanical characteristic is necessary. It has been suspected that water-soluble substances such as protein (Soda et al., 1981) or polysaccharides (Obinata et al., 1991) are responsible for the stickiness of buckwheat dough, but no obvious relationship between their components and the stickiness of dough has yet been reported.

Buckwheat is processed into noodles which has a unique mouthfeel and mechanical characteristics when compared to wheat noodles (Ikeda and Asami, 2000). This mouthfeel is one of the most important qualities factors of buckwheat noodles. Ikeda et al. (1997) suggested that protein and starch contents were responsible for the textural characteristics of heated buckwheat dough and they have continued studying protein functionality (Ikeda et al., 1999; Ikeda et al., 2002). In Japanese white wheat noodles, however, it was considered that starch functionality was mainly responsible for noodle texture. Subjective assessment values of noodle texture were associated with starch properties such as peak viscosity of wheat flour using a rapid-visco analyzer (RVA) together with an α-amylase inhibitor (Batey et al., 1997) and swelling volumes of starch, flour and wholemeal flour (Crosbie, 1991; Crosbie et al., 1992).

In this study, we investigated the correlation between mechanical characteristics of buckwheat dough or boiled dough and the constituent properties of buckwheat flour among 12 varieties. Moreover, we made a comparison of the starch properties between the products from 2001 and 2002 to check the consistency of the varietal difference.

MATERIALS AND METHODS

Correlative analysis between mechanical characteristics of dough and flour properties

(1) Materials

Common buckwheat varieties (*Fagopyrum esculentum* Moench cv. 'Kitawasesoba', 'Kitayuki', 'Hokkai No. 3', 'Hokkai No. 5', 'Hokkai No. 6', 'Mekei No. 15', 'Mekei No. 16', 'Shinanonatsusoba', 'Sumchanka', 'Green Flower', 'PGR2273', 'PGR5830') were sown in June 7, 2002 and normally cultivated in the experimental field of the National Agricultural Research Center for Hokkaido Region, Japan. The mature plants were harvested sepa-

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rately and the achenes were dried in the field. The achenes were completely dehulled using a dehuller (SP-X, Kokko Inc.) and the grain was then milled with a steel millstone (FC-120, Kokko Inc.) to make whole-grain flour. The flour was sifted through 60 mesh sieve.

(2) Toughness and hardness of dough

The original water content of the flour was determined beforehand by drying a portion of the sample for 1 hr at 135°C. Thirty gram of flour was placed into a polyethylene bag and mixed with an appropriate amount of distilled water to make dough of 42% water content. The dough was kneaded by hand for 1 min and then rolledout with a manual roller to a 2.5 mm thickness.

The toughness and hardness of dough were assessed using a rheometer (Yamaden Inc.) as shown in Fig. 1. A cylindrical plunger (ϕ5 mm) was run into the dough disk (2.5 mm thick, ϕ28 mm) with a 5 mm/sec speed and the resulting load on the plunger was recorded. The distortion percentage (distance from the surface of the dough to the breaking point/thickness of the dough) and the load on plunger at the breaking point were defined as toughness and hardness of dough respectively. All measurements were made in a constant temperature room at 20°C. The dough was prepared in four replications and five disks were made from each dough sample, i.e. the measurements were conducted twenty times for each variety.

(3) Hardness of boiled dough

Disks of dough (2.5 mm thick, ϕ28 mm), prepared as described above, were cooked in boiling water for 2.5 min, then cooled in water at 20°C for 2.5 min. The residual water attached on the surface of the boiled dough was blotted with a paper towel and the hardness of the boiled dough was assessed using a rheometer (Yamaden Inc.) as shown in Fig. 2. A cylindrical plunger (ϕ5 mm) went down and up once at a speed of 5 mm/sec until the boiled dough was distorted to 80% of its previous form. The maximal load on the plunger was recorded as the hardness of boiled dough. All measurements were made in a constant temperature room at 20°C. The dough was prepared in four replications and five disks were made from each dough samples, i.e. the measurements were conducted twenty times for each variety.

(4) Gelatinization of flour

An appropriate amount of flour was mixed with 25 ml of water to make a 8% (w/w) flour suspension. The suspension was stirred at 960 rpm for the first 10 sec and then at 160 rpm for the remainder of the analysis period. The gelatinization property of the mixture was measured

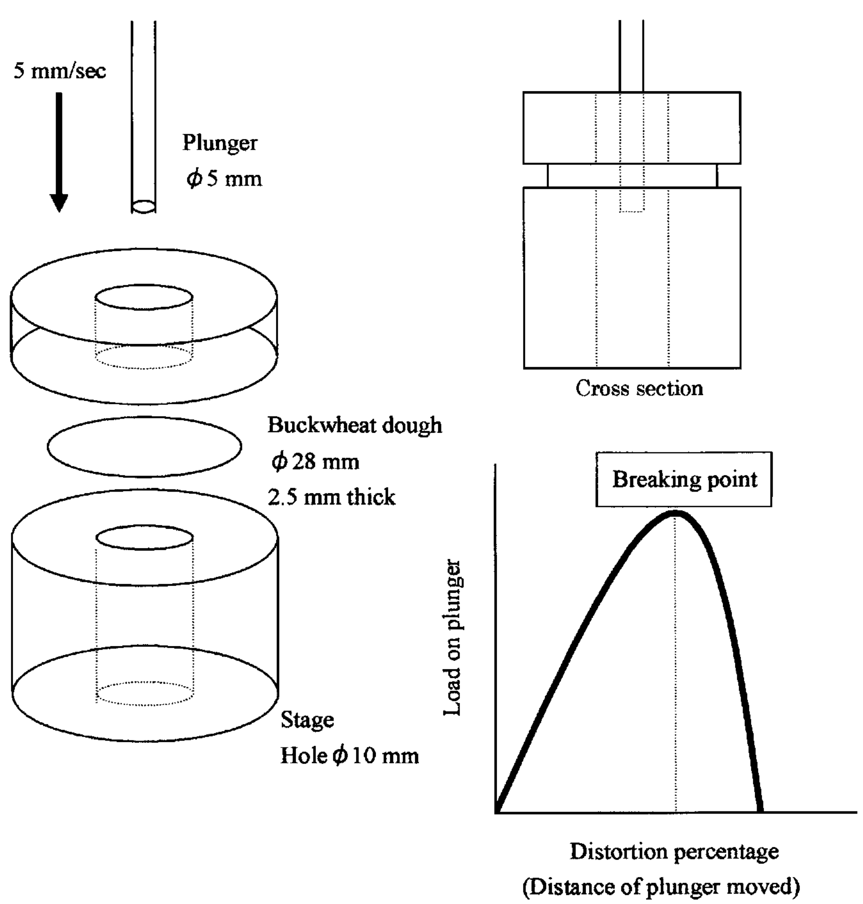

Fig. 1. The measurement of mechanical characteristics of buckwheat dough. The hardness of dough was evaluated with the load on plunger at the breaking point. The toughness of dough was evaluated with the distortion percentage at the breaking point.

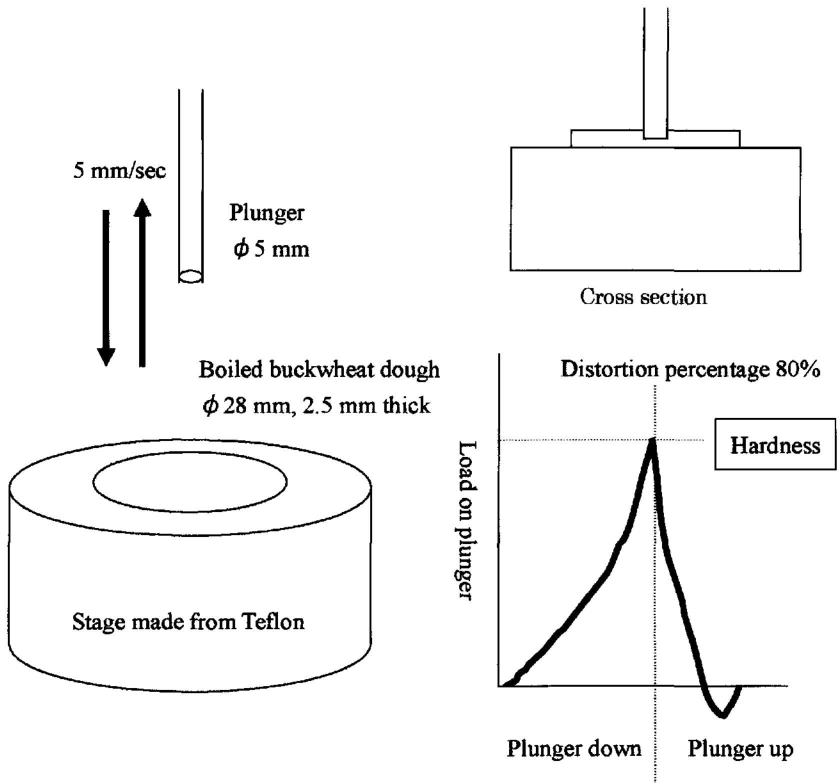

Fig. 2. The measurement of hardness of boiled buckwheat dough. The hardness of boiled dough was evaluated with the maximum load on plunger when the boiled dough was pressed to 80% distortion.

using a rapid-visco analyzer (RVA). The temperature profile for the standard analysis of starch was as follows: held at 50°C for 60 sec, heated to 95°C at 0.203°C/sec for 222 sec, held at 95°C for 180 sec, cooled to 50°C at 0.197°C/sec for 228 sec, and held at 50°C for 120 sec. Peak viscosity (maximal hot paste viscosity) and final viscosity (the viscosity at the end of the test) were measured as the RVA parameters. The measurements were replicated three times and all values were expressed in RVA unit (RVU).

(5) Flour-swelling power and starch-swelling power

Buckwheat starch was isolated using the method of Qian et al. (1998) with some small alterations. Ten grams of flour was suspended in 30 ml of 0.2% (w/v) NaOH and incubated in a water bath at 45°C for 45 min. The mixture was centrifuged at 3,000×g for 10 min and the supernatant was discarded. The sediment was re-suspended in 20 ml of 0.2% NaOH and incubated again in a water bath at 45°C for 45 min. The mixture was centrifuged at 3,000×g for 10 min again and the supernatant and the top yellow protein layer was removed. The white starch layer was then re-suspended in distilled water, centrifuged at 3,000×g for 10 min and the top yellow protein layer was again removed. This rinsing procedure was replicated four times. The sediment was re-suspended in distilled water, adjusted to pH 6.5–7.0 with 0.1 N HCl and centrifuged. The sediment was rinsed with distilled water twice, with ethanol twice, with acetone once and centrifuged. The sediment was re-suspended in distilled water and screened through a sieve (45 μm). After the sedimentation of the screened starch, the supernatant was discarded and the sediment was air-dried.

Swelling power was measured by the method of Fu et al. (1998) with some alterations. Approximately 50 mg of flour or starch was weighed into a pre-weighed tube (2 ml polypropylene microcentrifuge safe-lock tube, Eppendorf Inc.) and 1.5 ml of distilled water was added. The tube was then capped and mixed on a rotator (RVM-2048, Iwaki Inc.) for 30 min at 20 rpm. Then the tube was placed into a thermomixer (Thermomixer comfort, Eppendorf Inc.) and gelatinized at 95°C for 30 min with shaking at 1,400 rpm. After gelatinization, the tube was placed into a water bath (approximately 20°C) for 10 min and then centrifuged at 15,000×g for 10 min at 20°C. The supernatant was removed by suction and the tube with the sediment gel was weighed. The swelling power was calculated as the weight of sediment gel divided by the dry weight of the original sample. The moisture content of the original sample had been measured by drying a portion of the sample at 135°C for 1 hr. Swelling power measurements were replicated three times for each flour or starch sample.

(6) Crude protein and water-soluble protein content

Crude protein content was determined using a NC analyzer (NC-900, Sumika Inc.) by measuring nitrogen gas from the combusted sample. Approximately 10 mg of flour was used for each measurement. The crude protein content was calculated by multiplying the nitrogen content by the nitrogen-to-protein conversion factor (6.25).

Water-soluble protein content was determined by the Kjeldahl method. Approximately 1 g of flour was put into a 15 ml tube and mixed with 10 ml of distilled water on a rotator for 30 min at room temperature. The mixture was centrifuged at 3,000×g for 10 min and the supernatant was collected into a Kjeldahl tube. The sediment was further extracted twice with 10 ml of distilled water and the supernatants were collected into the same Kjeldahl tube. The collected supernatant was dried in a drying oven at 80°C for 1 day and then decomposed by H_2SO_4 and H_2O_2 at 420°C. The nitrogen content of the decomposed sample was measured by the Kjeldahl method with a Kjeltec Auto 1030 analyzer (Tecator Inc.). The protein content was calculated by multiplying the nitrogen content by the nitrogen-to-protein conversion factor (6.25). The measurements were replicated three times for the crude and water-soluble protein contents.

(7) Crude fat content

About 6 g of flour was put into a cylindrical filter paper and extracted by diethyl ether for 1.5 hr using a Soxtec 1043 extraction unit (Tecator Inc.). The residual diethyl ether in the crude fat was removed in a drying oven at 135°C for 24 hr and then weighed. The crude fat content was calculated as the weight of the extracted crude fat divided by the dry weight of the original flour sample. The measurements were replicated three times.

(8) Statistical analysis

In the above characteristics, the replications were averaged for each variety and the average values were used for the correlation analysis. The correlation coefficient was calculated by using Excel Statistics (Microsoft Inc.) between every characteristics and a correlation matrix was made.

Comparison of gelatinization property between the products of 2001 and 2002

In 2001, all varieties except for 'Green Flower' were cultivated in the same experimental field as 2002. The buckwheat achenes produced in 2001 were milled using a test mill (Bravender Duisburg Nr181513 Type 279002, Brabender Inc.) and the flour was sifted through 60 mesh sieve. The flour was kept in a freezer at –30°C until analyzed.

The RVA peak viscosity and starch-swelling power with these samples were measured as described above and the correlations of RVA peak viscosity and starch-swelling power between the products of 2001 and 2002 were then investigated.

RESULTS AND DISCUSSION

Variation among samples tested

The ranges of the measured characteristics are shown in Table 1. The hardness of the dough was highest in 'Shinanonatsusoba' (the load on plunger when the dough broke=151 gf), 'Sumchanka' (151), 'Kitayuki' (150) and 'Hokkai No. 6' (145) and lowest in 'PGR2273' (126), 'Hokkai No. 3' (129) and 'Green Flower' (131). On the contrary, the toughness of the dough was highest in 'Green Flower' (the distortion percentage when the dough broke=105%), 'PGR2273' (101) and 'Hokkai No. 3' (98.4) and lowest in 'Hokkai No. 6' (73.2), 'Sumchanka' (74.7) and 'Kitayuki' (74.8). The toughness of the dough was found to be negatively correlated with the hardness of the dough ($r=-0.861^{**}$, Table 2), i.e. the harder the dough, the more fragile.

The hardness of the boiled dough was highest in

Table 1. Ranges of values for measurements carried out on samples from 12 varieties grown at Memuro, Hokkaido, Japan, in 2002.

Character	Unit	Minimum (Variety)			Maximum (Variety)	
Load on plunger when the dough broke (Hardness of dough)	gf	126	(PGR2273)	~	151	(Shinanonatsusoba)
Distortion percentage when the dough broke (Toughness of dough)	%	73.2	(Hokkai No. 6)	~	105.0	(Green Flower)
Hardness of boiled dough	gf	971	(PGR2273)	~	1230	(Mekei No. 16)
RVA peak viscosity of flour	RVU	65.33	(Mekei No. 16)	~	110.39	(Hokkai No. 3)
RVA final viscosity of flour	RVU	98.89	(Mekei No. 16)	~	178.14	(Hokkai No. 3)
Starch swelling power	g gel/g starch	14.38	(Mekei No. 16)	~	18.90	(PGR2273)
Flour swelling power	g gel/g flour	8.43	(Mekei No. 16)	~	10.63	(Hokkai No. 3)
Crude protein content	g/100 g DM	12.2	(Shinanonatsusoba)	~	14.9	(Green Flower)
Water-soluble protein content	g/100 g DM	7.1	(Shinanonatsusoba)	~	9.5	(Green Flower)
Crude fat content	g/100 g DM	2.51	(Sumchanka)	~	3.03	(Green Flower)

'Mekei No. 16' (1230 gf), 'Sumchanka' (1149) and 'Mekei No. 15' (1146), and low in 'PGR2273' (971), 'Hokkai No. 3' (988) and 'Kitawasesoba' (1000). The hardness of the boiled dough had a significant relation to the hardness of dough (r=0.655*, Table 2) and the toughness of the dough (r=−0.803**, Table 2). Soft and less fragile dough tended to produce a soft boiled dough.

The RVA peak viscosity was highest in 'Hokkai No. 3' (110.39 RVU) and 'Sumchanka' (102.06), and lowest in 'Mekei No. 16' (65.33), 'Mekei No. 15' (79.00) and 'Shinanonatsusoba' (83.03). The final viscosity was found to be parallel to the peak viscosity; it was highest in 'Hokkai No. 3' (178.14 RVU), 'Sumchanka' (158.78), and lowest in 'Mekei No. 16' (98.89), 'Mekei No. 15' (125.67) and 'Shinanonatsusoba' (130.50). The correlation coefficient between the peak and final viscosity was 0.992** (Table 2).

The starch-swelling power was highest in 'PGR2273' (18.90 g gel/g starch) and 'Hokkai No. 3' (18.67), and lowest in 'Mekei No. 16' (14.38), 'Shinanonatsusoba' (16.45) and 'Mekei No. 15' (17.07). The flour-swelling power was nearly parallel to the starch-swelling power; it was highest in 'Hokkai No. 3' (10.63 g gel/g flour) and 'Sumchanka' (10.55), and lowest in 'Mekei No. 16' (8.43), 'Shinanonatsusoba' (9.28) and 'Mekei No. 15' (9.51). The flour-swelling power was found to be smaller than the starch-swelling power. We suggest that this difference was caused by the presence of non-swelling substances such as protein, fiber and fat in the flour. In addition, these impurities might inhibit starch gelatinization. Nevertheless, a strong correlation could be observed between the starch-swelling power and flour-swelling power (r=0.962**, Table 2). We therefore recommend that flour-swelling power can be used to evaluate samples for their starch-swelling property.

The crude protein content was found to be maximal in 'Green Flower' (15.04%) and minimal in 'Shinanonatsusoba' (12.35). In most varieties, it was approximatley 13%. The water-soluble protein content was maximal in 'Green Flower' (9.60%), and minimal in 'Shinanonatsusoba' (7.20). The amount of water-soluble protein as compared to crude protein ranged from 56.24% ('Hokkai No. 6') to 66.08% ('Hokkai No. 3'). Javornik et al. (1981) separated buckwheat protein into fractions comprising 18.2% albumins, 43.3% globulins, 0.8% prolamins, 22.7% glutelins and 15.0% in extractable nitrogen compounds. The water-soluble protein content found in this experiment approximately corresponded to the combined content of the albumins and globulins.

The crude fat content was found to be maximal in 'Green Flower' (3.03%) and minimal in 'Sumchanka' (2.51%).

Relationships between mechanical characteristics of dough and constituent properties

The correlation coefficients between the hardness or the toughness of the dough and constituent properties are shown in Table 2. The hardness and toughness of the dough had a significant correlation with the water-soluble protein content (r=0.684* and r=0.775** respectively). The dough became softer and less fragile as the flour contained more water-soluble protein. The other characteristics had no significant correlation with the other mechanical characteristics of dough.

Soda et al. (1981) suggested that acid-precipitated water-soluble buckwheat protein had a 10–40 times higher intrinsic viscosity than soybean globulin. They suspected a relationship between buckwheat protein and the fragile-

Table 2. Correlation table of values for measurements carried out on samples from 12 varieties grown at Memuro, Hokkaido, Japan, in 2002.

Character	RVA peak viscosity	RVA final viscosity	Starch swelling power	Flour swelling power	Crude protein	water-soluble protein	Crude fat	Hardness of dough	Toughness of dough
RVA final viscosity of flour	0.992**								
Starch swelling power	0.800**	0.808*							
Flour swelling power	0.889**	0.879**	0.962**						
Crude protein content	−0.012	−0.054	0.361	0.332					
Water-soluble protein content	0.295	0.237	0.415	0.473	0.819**				
Crude fat content	−0.097	−0.131	0.261	0.147	0.448	0.509			
Load on plunger when the dough broke (Hardness of dough)	−0.279	−0.249	−0.360	−0.325	−0.467	−0.684*	−0.543		
Distortion percentage when the dough broke (Toughness of dough)	0.471	0.420	0.533	0.539	0.518	0.775**	0.568	−0.861**	
Hardness of boiled dough	−0.729**	−0.728**	−0.791**	−0.775**	−0.186	−0.439	−0.371	0.655*	−0.803**

*,**: statistically significant at the 1% and 5% level, respectively.

ness of buckwheat dough. On the other hand, Obinata et al. (1991) proposed that polysaccharides were responsible for the viscosity of the water extracts from buckwheat flour. However, a simple measuring method for polysaccharides has not been established yet. Therefore, the water-soluble protein content seems to be initially a better index for the improvement of mechanical characteristics of buckwheat dough.

The water-soluble protein content was found to have a significant correlation ($r=0.819^{**}$) with crude protein content (Table 2). Since crude protein content can be measured using a small amount of flour, this might be a good for the first step for noodle quality improvement.

Relationships between hardness of boiled dough and constituent properties

The hardness of the boiled dough had significant relationships to starch properties such as RVA peak viscosity ($r=-0.729^{**}$), RVA final viscosity ($r=-0.728^{**}$), starch-swelling power ($r=-0.791^{**}$) and flour-swelling power ($r=-0.775^{**}$). The boiled dough became softer as the RVA peak or final viscosity became higher and the starch- or flour-swelling power became larger. Since the flour-swelling power could be measured with only 50 mg of flour and with a shorter analysis time than RVA, it should be a good index for the selection of starch properties in early generations of buckwheat breeding.

Ikeda et al. (1997) suggested that the starch or amylopectin content positively correlated to the springiness of heated dough and the protein content negatively correlated to the springiness and chewiness of heated dough. In their further studies, they mainly focused on the role of protein in relation as it affected the mechanical characteristics of heated dough (Ikeda et al., 1999; Ikeda and Asami, 2000; Ikeda et al., 2002). In this experiment, however, the protein content did not have a significant correlation with the hardness of boiled dough. We suspected that this difference was caused by the variation of protein content among the samples. The range of protein content in this experiment was smaller than that in Ikeda et al. (1997). Therefore the effect of protein content might be obscure in our study.

In the case of wheat, the starch properties, such as swelling power and RVA viscosity have a strong correlation with the subjective assessment value of Japanese noodle texture (Crosbie, 1991; Crosbie et al., 1992; Batey et al., 1997). Although the textural preference in Japanese wheat noodles is substantially different from that found in Japanese buckwheat noodles, we suspect that differences in starch properties can be used to predict buckwheat noodle texture. However, consumer preference for buckwheat noodle texture should be surveyed before any breeding efforts are expended.

Comparison of starch properties between the products of 2001 and 2002

The correlation diagram of the RVA peak viscosity between the products of 2001 and 2002 is shown in Fig. 3. The correlation coefficient was significant ($r=0.762^{**}$) even though the samples were powdered with a Brabender test mill in 2001 and with a steel millstone in 2002. However, the RVA peak viscosities in 2001 were generally higher than those found in 2002. This difference could be due to the difference of the milling method used or to the year to year environmental effects. The yield rate of the Brabender test mill was lower than that of the steel millstone. The flour milled with the Brabender test mill also had a lower protein content than that milled with the steel millstone; conversely, it might have contained a higher amount of starch (data not shown).

A correlation diagram of the starch-swelling power between the products of 2001 and 2002 is shown in Fig. 4. The correlation coefficient was very high ($r=0.984^{**}$) and was higher than that of RVA viscosity. The starch-swelling power might be a heritable character since the varietal differences were highly consistent between the samples produced in 2001 and 2002. However, the starch-swelling powers in 2001 were generally higher than those in 2002. We suspected that some impurities still might have remained in the isolated starch and affected the results of starch-swelling power. It was technically impossible to isolate pure starch from buckwheat flour. As we described above, the flour samples of 2001 contained smaller amount of protein than those of 2002, therefore the isolated starch from the samples of 2001 might contain smaller amount of impurities.

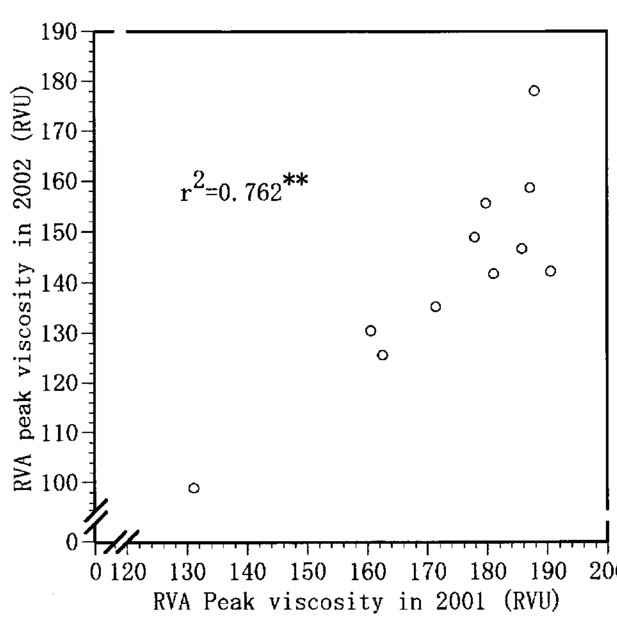

Fig. 3. Comparison between the buckwheat flour products of 2001 and 2002 in RVA peak viscosity.

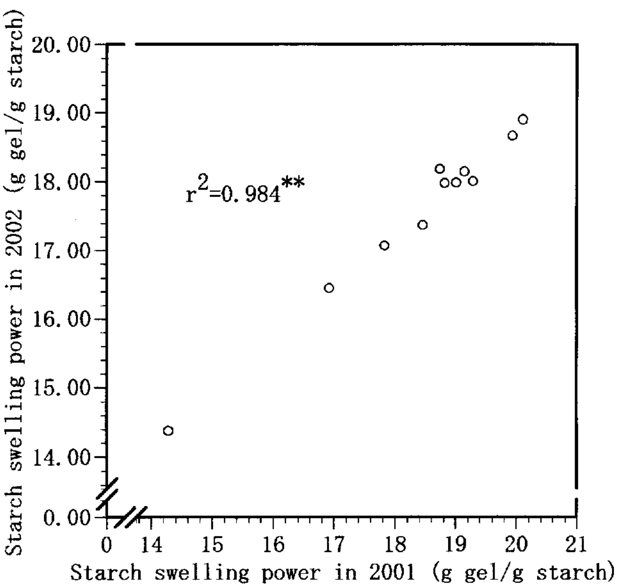

Fig. 4. Comparison between the products of 2001 and 2002 in starch-swelling power.

CONCLUSION

The results suggest that a flour-swelling power test had a good possibility to be used to evaluate buckwheat flour for the ability to make improved buckwheat noodles. The flour-swelling power was found to have a significant correlation with the hardness of boiled dough. The test is simple, rapid and inexpensive. It only needs small amount of wholegrain dehulled buckwheat flour and it would be applicable for individual plants as well as for breeding lines. In addition, the water-soluble protein content had a significant correlation with the fragileness of dough. The water-soluble protein content also had a significant correlation with crude protein content. The crude protein content can be easily measured with a small amount of sample by a NC analyzer and it might be a good index to be first used in the improvement of the mechanical characteristics of the dough.

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Changes in the solubility of the minerals in buckwheat noodles occurring by processing, cooking and enzymatic digestion

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ABSTRACT

Changes in the solubility of seven essential minerals of buckwheat noodles on cooking and the release of the mineral by enzymatic digestion were analyzed. The minerals subjected for analysis were zinc, copper, manganese, calcium, magnesium, potassium and phosphorus. When raw noodles prepared from buckwheat flour were cooked, approximately 20 to 40% of the minerals in the raw noodles were found to leak into water soak during cooking. In addition, about 20% of protein in raw noodles was found to leak into water soak. There was a remarked variation in the proportion of the amount of some minerals to their original total contents, i.e., zinc, copper, manganese, calcium and magnesium, released after enzymatic digestion, and cooking noodles. High proportions of zinc, copper and potassium were released as a soluble form from cooked buckwheat noodles after enzymatic digestion. Nutritional implications of the present findings were discussed in relation to traditional dietary customs of buckwheat products.

INTRODUCTION

Buckwheat (*Fagopyrum* spp.) is an important food in some areas 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 a low glycemic index (Jenkins, 1981; Foster-Powell and Miller, 1995) and beneficial effect on cholesterol metabolism (He et al., 1995), increasing attention has been currently paid to buckwheat as a functional food (Bonafaccia and Kreft, 1998; Mazza, 1998). Buckwheat flour contains various kinds of essential nutrients including protein, starch and essential minerals. We have conducted studies on the nutritional function of essential minerals in buckwheat and its products (Ikeda and Yamashita, 1994; Ikeda, 1996; Ikeda et al., 2001, 2002). The nutritional characteristics of essential minerals in buckwheat, however, have not been fully clarified.

On the other hand, there are many buckwheat products and various buckwheat dishes around the world (Kreft et al., 2003). Buckwheat noodles are the most popular buckwheat food product in Japan (Ikeda and Ikeda, 2003). In Japan, there is a traditional dietary custom of drinking the hot water (soba-yu) which is left after cooking the buckwheat noodles. However, any scientific basis for such a dietary custom has not yet been fully elucidated (Nagatomo, 1984; Ikeda and Shimizu, 1993).

Current evidence (Suzuki and Wada, 1994) has shown that sixteen or more kinds of minerals are essential for human nutrition. Each essential mineral has many diverse physiological functions. Recommended dietary allowances for essential minerals have been established in many countries in recent years. In Japan, the recommended dietary reference intakes for essential minerals for Japanese has been recently established (MHWJ, 1999). The evaluation of diets for the adequacy of minerals requires knowledge on both the amount and the bioavailability of the minerals for intestinal absorption. Thus clarifying both the amount of minerals in the food stuff and their bioavailability is a subject of great interest. Although minerals are widely distributed in foods, variations in the bioavailability of minerals have been found among different foods (Yasumoto, 1994). The bioavailability of food minerals has been considered to be affected by various factors, such as proteins, phytic acid, other minerals, and dietary fiber (Yasumoto, 1994). These findings suggest that the chemical form in which dietary minerals are presented to the intestinal absorptive cells may have a profound influence on the bioavailability of the minerals.

The present study was undertaken to analyze the composition of essential minerals, i.e., zinc, copper, manganese, calcium, magnesium, potassium and phosphorus, in buckwheat noodles, and to clarify changes in the amount of seven minerals in their soluble form after in vitro enzymatic digestion and cooking of buckwheat or processing it into noodles.

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MATERIALS AND METHODS

Materials

Fresh pure flour of common buckwheat (*Fagopyrum esculentum* Moench cultivar. Kitawase-soba, harvested in Horokanai, Hokkaido) with a milling yield of approximately 93% was obtained from a local dealer and used immediately. The buckwheat noodles were prepared according to common practice in our laboratory. Fig. 1 shows a diagram of the preparation and cooking of the buckwheat noodles. One hundred grams of buckwheat flour was first mixed with approximately 45 ml of hot distilled water and then buckwheat noodles were prepared by hand. The prepared noodles were cooked in 500 ml of boiling water for 3 min. Then, additional cold water (100 ml) was added to the suspension of the noodles and the noodles were cooked for 3 min. further. After cooking, the noodles were separated from the water. The noodles were then fully washed in an excess of cold water. Fig. 1 also shows changes in weight during the preparation of buckwheat noodles. Fig. 2 shows then buckwheat noodles as prepared for this study.

Determination of essential minerals and protein

Seven essential minerals, i.e., zinc, copper, manganese, calcium, magnesium, potassium and phosphorus were assayed in this study. These essential minerals, except for phosphorus, were determined from the buckwheat flour samples with a Hitachi Z-5300 polarized Zeeman atomic absorption spectrophotometer (Hitachi Ltd., Hitachi-naka, Japan). Phosphorus was assayed using the colorimetric method of Fiske and Subbarow (1925). Prior to determining the total content of these essential minerals, the buckwheat flour samples were wet-ashed with sulfuric acid and 30% hydrogen peroxide. The protein content was analyzed by the micro-Kjeldahl method (AOAC, 1984) (N×6.25).

In vitro proteolytic digestion

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

RESULTS AND DISCUSSION

Essential mineral composition in buckwheat noodles and its nutritional contribution

Table 1 shows the composition of the seven essential minerals, i.e., zinc, copper, manganese, calcium, magne-

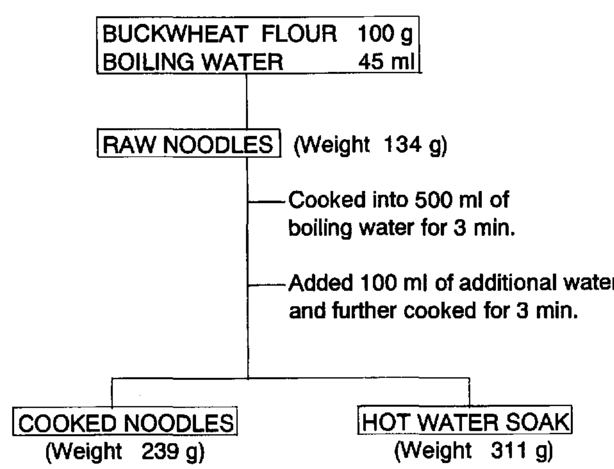


Fig. 1. Diagram of the preparation and cooking of buckwheat noodles.

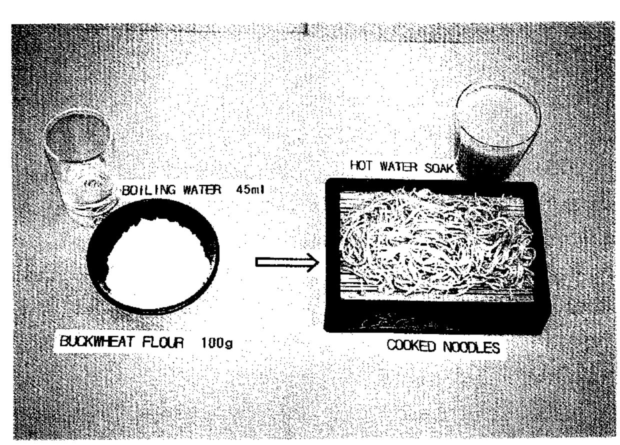


Fig. 2. Buckwheat noodles prepared.

sium, potassium and phosphorus, and total protein content in raw noodles, cooked noodles and hot cooking water. There was a difference in the content of all of the minerals between the raw noodles and the cooked noodles (Table 1). This finding suggests that all the minerals may have leaked into the cooking water on cooking. Considerable amounts of the minerals were subsequently found in the cooking water (Table 1). A decrease in the content of all seven minerals in buckwheat noodles was observed upon cooking (Table 1).

The present study showed that buckwheat noodles contain various amounts and kinds of essential minerals (Table 1). In this connection, recommended dietary allowances for essential minerals have recently been established in many countries. MHWJ established the recommended dietary reference intake for essential minerals (RDA) for Japanese (1999). Based on both the analytical data in Table 1 and the RDA (MHWJ, 1999), we evaluated the nutritional contribution of cooked noodles prepared from the buckwheat flour (Fig. 2) for the seven minerals. Our evaluation suggested that one dish, i.e., 239 g of cooked buckwheat noodles prepared from 100 g of buckwheat flour, can provide approximately 10 to 13% of the zinc, approximately17 to 22% of the copper, approximately 34 to 45% of the manganese, approximately 1% of the calcium, approximately 58 to 77% of the magnesium, approximately 8% of the potassium and approximately 39% of the phosphorus required for the RDA. Thus buckwheat noodles after cooking can provide a good potential source of zinc, copper, manganese, magnesium and phosphorus, but do not provide any significant amounts of calcium and potassium, for the RDA.

On the other hand, a considerable amounts of the seven minerals were subsequently found in the cooking water: approximately 18 to 38% of the minerals from the raw buckwheat noodles leached into the water upon cooking (Table 1). In Japan, there is a traditional dietary custom of drinking the hot cooking water left after cooking the buckwheat noodles, "soba-yu". It was believed that the cooking water may contain some required nutrients (Nagatomo, 1984), although the scientific basis for such a dietary custom had not been fully elucidated (Ikeda and Shimizu, 1993). The present finding shows that a considerable amount of the seven minerals evaluated in raw buckwheat noodles leached into the hot cooking water during cooking of the buckwheat noodles.

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

These findings suggest that the hot cooking water (soba-yu) after cooking of the noodles contain nutrients such as protein and some required minerals (Table 1). These findings (Table 1) may provide the basis for such a dietary custom.

The essential minerals released after in vitro enzymatic digestion of the buckwheat noodles and hot cooking water

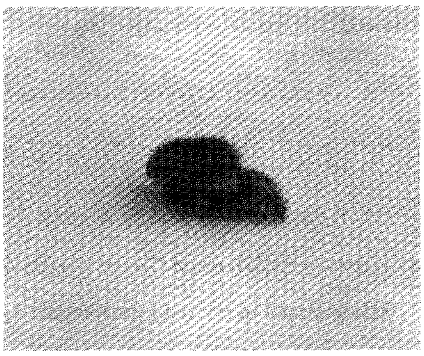
Table 2 shows the amount (and the proportions to their original total content) of each essential mineral, i.e., zinc, copper, manganese, calcium, magnesium, potassium and phosphorus, released as soluble forms after amylase, pepsin plus pancreatin digestion of the buckwheat noodles and of the hot cooking water. A large variation in the proportions of the minerals released upon enzymatic digestion of the raw noodles was found among the seven minerals (Table 2): a higher proportion which was released after enzymatic digestion was found for zinc and potassium, whereas a lower proportion was found for calcium and manganese. Cooking increased the proportions of the

Table 1. Composition of seven essential minerals and total protein in buckwheat noodles and hot water soak[1)].

Foods examined	(A) Weight	Zn	Cu	Mn	Ca	Mg	K	P	Protein
	g	mg/EW[2)]							g/EW[2)]
Raw noodles	134	1.93	0.48	1.89	11.7	229	395	467	10.93
		(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)
Cooked noodles	239	1.14	0.31	1.34	8.4	184	163	276	6.63
		(59)	(65)	(71)	(72)	(80)	(41)	(59)	(61)
Hot water soak	311	0.66	0.15	0.37	3.0	45	151	85	2.35
		(34)	(31)	(20)	(26)	(20)	(38)	(18)	(22)

[1)] Values are means on a wet weight basis [n=4]. Values in parenthesis indicate per cent of each content of mineral and protein of the cooked noodles and the water soak to each content of the raw noodles when each content of mineral and protein in the raw noodles were taken as 100%.
[2)] EW, each weight indicated in (A) within the same line.

a



b

Fig. 1. a: Leaf weevil adults (female and male) on buckwheat leaves and b: Adults in mating (♀ carrying the ♂)

exarate type of pupae is creamish coloured and this stage lasts for 9.3 ± 1.02 days. The total life span varied from 42–56 days in the summer from egg to adult emergence. Adults were observed to be the over-wintering stage of the pest. On review of the literature, no report pertaining to weevil damage in the buckwheat crop could be found. Other species of weevils, *Sagra femorata* (Drury), *S. nigrita* Olivier and *Cypericerus emerginatus* FST. with similar nature of damage on lablab and beans have been reported by Tandon et al. (1975), Nair (1986) and Thakur et al. (1996), but their life cycle differed from *S. kumaoensis*. The pupal period in the case of *S. nigrita* and *S. femorata* is very long, i.e. 4–5 months and 5–6 months, respectively, whereas it is very short 4–6 days in the case of *C. emarginatus*. Moreover no gall formation was observed in case of *S. kumaoensis* as is the case with *S. nigrita*, *S. femorata* and *C. emarginatus*. The weevil population under field conditions could be managed by applying fenvalerate dust @ 20 kg per hectare at the time of sowing or by spraying the crop with synthetic pyrethroids *viz.* deltamethrin 0.0028% or fenvalerate 0.01% or cypermethrin 0.0075% immediately after the germination of the crop.

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The 9th International Symposium on Buckwheat at Prague

Symposium Organisational Structure

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Zhuanhua Wang (CHN)
Friedrich J. Zeller (GER)
Gunilla Wieslander (SWE)
Dan Norback (SWE)

Invitation

Dear buckwheat friends,

I have a great opportunity to invite you to the 9th International Symposium on Buckwheat 2004 that will be held in Prague, the capital of the Czech Republic widely known as "The Heart of Europe".

The Czech Republic has had long history in cultivation of buckwheat and its use as a nutritional foodstuff. The oldest records about buckwheat are dated back to the 12th century. Buckwheat had been a staple crop for the major part of population over many centuries and a lot of family businesses were based on buckwheat growing and processing yet in the 19th century. During that time buckwheat became a traditional food crop in many regions throughout the Czech Lands and according to different annals other Middle European countries enjoyed similar popularity in buckwheat cultivation as well.

After a few past centuries of gradual decline in buckwheat production the end of the last century has started its revival. The interest in growing buckwheat increases especially as one of the most important crops in organic farming. Currently the Czech Republic is among the most important producers of organically farmed buckwheat in Europe.

This crop has proven its broad potential in many scientific and non-scientific areas and takes significant part in their diversification. Buckwheat is highly nutritious crop with significant influence on health and human diet. Buckwheat has numerous uses in food industry and also in feedstuff and pharmaceutical industry or cosmetics.

It has been an honour for us and for the whole Czech buckwheat community to be in charge of hosting such international event. I and all members of the organising committee believe that you will enjoy the meeting filled with interesting scientific pieces of knowledge and practical information applicable to any place in the "Buckwheat World".

Anna Michalova
Organising Committee

The Venue

Czech University of Agriculture

All the lectures and poster sessions will take place within Czech University of Agriculture in Prague. Its modern Congress Centre, facilities, location and academic environment make it the ideal centre for self-contained scientific conferences and symposia.

Preliminary Programme

Date	Morning–Early Afternoon	Late Afternoon–Evening
August 18 Wednesday	Registration	IBRA Committee Meeting
August 19 Thursday	Opening Ceremony, Presentations and Lectures	Welcome Party
August 20 Friday	Presentations and Lectures	Sightseeing Tour of Prague
August 21 Saturday	Presentations and Closing, IBRA Meeting	Social Evening at Pilsner Urquell
August 22 Sunday	Excursion	Excursion

Forms of Presentation

There will be several forms of presentation at the Symposium:

A) Presentation

Invited Speakers - Special lectures presented by reputable experts in the field (20 minutes presentation)

Other lectures - 15 minutes

B) Paper contribution (also available for those who are not able to attend the symposium but are willing to participate and deliver a paper to be included in Symposium Proceedings)

C) Poster presentation

Registration

The deadline for Early Registration and Symposium Accommodation reservation is **31 March 2004.**

● Full details of the prices for registration and accommodation are printed on the enclosed forms F1-F3 of the second announcement. Completed forms, with full payment, can be posted, or faxed to:

IX. International Symposium on Buckwheat
Research Institute of Crop Production
Drnovska 507
16106 Prague - Ruzyne
Czech Republic

Phone: +420 233 022 406
Fax: +420 233 022 286
E-mail: isb2004@vurv.cz

Alternatively, registration and accommodation can be booked on-line at secure website **http://www.vurv.cz/buckwheat**

List of Important Dates

31 March 2004 Deadline for Early Registration and Accommodation Bookings	*19 August 2004* Opening Ceremony and Welcome Party
31 March 2004 Deadline for all Paper Submissions	*20 August 2004* Social Evening at Pilsner Urquell
1 July 2004 No refund of registration fees	*22 August 2004* Scientific Excursion

A letter from the president of IBRA

I am very happy to publish the volume 20 of the Fagopyrum. Since the first volume of Fagopyrum was issued at Ljubljana by Prof. Ivan Kreft in 1981, the Journal Fagopyrum has progressed a great deal in respect to quantity and quality. Fagopyrum is the only international journal on buckwheat science. It includes all of the buckwheat-related sciences including ethnobotany, breeding, agronomy, physiology, biotechnology, nutrition, processing, pharmaceutical effectiveness, etc. The last four volumes (vol. 15 to vol. 19) of Fagopyrum contained 60 articles from 194 authors from 14 countries of the world. The cover pages have been well designed and are very attractive to the reader. However, we have to make a larger effort to publish more volumes each year and to increase subscribers around the world. We should also try ot get Fagopyrum to be a SCI registered-journal for the purpose of improving its quality. All IBRA members are responsible for the improvement of our Fagopyrum journal and thus should take part in the submission of both qualified articles and in the expansion of finance.

I give special thanks to Dr. Ohmi Ohnishi as editor-in-chief for his invaluable editing of the Fagopyrum for the last five years.

I hope that all members participate to the 9th International Symposium on Buckwheat at Prague in 2004. I do not doubt that the 9th ISB will be a very useful and wonderful meeting to give participants many memorials. I am looking forward to seeing all of you at Prague next year.

Dr. Cheol Ho Park
President
International Buckwheat Research Association

20th anniversary of FAGOPYRUM

It is my pleasure to inform the members of the IBRA that the journal FAGOYRUM is now celebrating its 20th anniversary. FAGOPYRUM was founded as the Buckwheat Newsletter in 1981 by Prof. Ivan Kreft, University of Ljubljana, Ljubljana, Yugoslavia.

It contained 6 short papers plus Abstracts and Bibliographies of papers (or books) published elsewhere. In the next year, it changed its name to FAGOPYRUM. FAGOPYRUM had a green front page cover beginning with volume 5 (1985) until vol. 7 in 1987. FAGOPYRUM then changed its style in vol. 8 (1988) where it had a colored front cover and contained 14 research papers, this is almost the same style as you see in vol. 20 (2003).

After difficulties in publication of FAGOPYRUM at the University of Ljubljana had arisen, Prof. T. Matano, Shinshu University, Ina, Japan began editing FAGOPYRUM and published volume 15 in 1998 after three years of no publication. At the 7th International Symposium on Buckwheat at Winnipeg, Canada in 1998, the issue of publication of FAGOPYRUM was discussed and the present editorial office was settled.

This is a brief history of the publication of FAGOPYRUM (Buckwheat Newsletter). In volume 20 (2003), three special invited papers have been included as was done in volume 10 (1990). We currently print 500 copies of FAGOPYRUM and distribute them to all of the IBRA members world wide. We hope that our IBRA members will increase in number and contribute more scientific papers in FAGOPYRUM.

Ohmi Ohnishi
Editor-in-Chief of FAGOPYRUM

INSTRUCTIONS TO AUTHORS

FAGOPYRUM accepts scientific papers, and information and bibliographies on buckwheat.

SCIENTIFIC PAPERS

Manuscript should be written in standard English and submitted in triplicate to the Editorial Office. Submission through E-mail is also acceptable.

Ohmi Ohnishi
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Plant Germ-plasm Institute
Graduate School of Agriculture, Kyoto University
Nakajo 1, Mozume-cho, Muko 617–0001, Japan
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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.

The manuscript should be arranged in the following order.

1. Title page (page 1)
   —Title (the title should be as short as possible, but should contain adequate information to indicate the contents)
   —Author's full name(s)
   —Affiliation(s)/Address(es)
2. Key words/Running head (not to exceed 50 letters including spaces) (page 2)
   —Key words (maximum of 5, in alphabetical order, suitable for indexing)
3. Abstract (brief and informative, not to exceed 250 words).
4. Main text
   —Introduction, Materials and Methods, Results, Discussion
   —The relative importance of headings and subheadings should be clear.
5. The approximate location of figures and tables should be indicated in the margin.
   —The use of footnotes should be avoided.
6. After the main text
   —Acknowledgements (also grants, support etc., if any) should follow the text and precede the references
7. References
   —The literature references should be arranged alphabetically, typed double spaced and in the text referred to as: author and year of publication, e.g. Budagovskaya (1998), (Inoue et al., 1998). Citation of personal communications and unpublished data should be avoided, unless abolutely necessary. Such citations should be in text appear only as (R. Brown, personal communication), and not in the reference list. To abbreviate titles of periodicals, refer to recent issues of FAGOPYRUM.

Follow the style shown below: Periodicals

Budagoskaya, N., 1998. Changes in the state of photoautotrophic and heterotrophic organs of buckwheat plants at iron deficiency and low pH. Fagopyrum 15: 1–7.

Inoue, N., M. Hagiwara, H. Y. Kim and T. Matano, 1998. A preliminary study for modeling seed production in common buckwheat. Fagopyrum 15: 35–41.

Books (edited by someone other than author of article)

Hattermer, H. and H. G. Gregorius, 1990. Is gene conservation under global climate meaningful? In: Jackson, M. T., B. V. Ford-Lloyd and M. L. Parry(eds.), Climatic Change and Plant Genetic Resources, pp. 158–166,

Bellhaven Press, London.

Books (identical author and editor)

Campbell, C. G., 1997. Buckwheat. IPGRI, Rome.

8. Tables

—Each table should be mentioned in the text.

—Each table should be typed on a separate page.

—Tables should be numbered with Arabic numerals, followed by the title.

—Horizontal rules should be indicated; vertical rules should not be used.

—Tables may be edited by the editor to permit more compact typesetting.

9. Figures

—Each figure should be mentioned in the text.

—Each figure should be numbered by Arabic numerals.

—Line drawings should be in a form suitable for reproduction without modification. Extremely small type should be avoided as figures are often reduced in size.

—Photographs should be supplied as black-and-white, high-contrast glossy prints. Color prints may be inserted at the author's own expense.

—Identify each illustration, on the back, by writing author's name and figure number.

INFORMATION and BIBLIOGRAPHY

Information (including short preliminary reports) and bibliographical contributions should be shorter than two printed pages, and will be published without being refereed.

sium, potassium and phosphorus, and total protein content in raw noodles, cooked noodles and hot cooking water. There was a difference in the content of all of the minerals between the raw noodles and the cooked noodles (Table 1). This finding suggests that all the minerals may have leaked into the cooking water on cooking. Considerable amounts of the minerals were subsequently found in the cooking water (Table 1). A decrease in the content of all seven minerals in buckwheat noodles was observed upon cooking (Table 1).

The present study showed that buckwheat noodles contain various amounts and kinds of essential minerals (Table 1). In this connection, recommended dietary allowances for essential minerals have recently been established in many countries. MHWJ established the recommended dietary reference intake for essential minerals (RDA) for Japanese (1999). Based on both the analytical data in Table 1 and the RDA (MHWJ, 1999), we evaluated the nutritional contribution of cooked noodles prepared from the buckwheat flour (Fig. 2) for the seven minerals. Our evaluation suggested that one dish, i.e., 239 g of cooked buckwheat noodles prepared from 100 g of buckwheat flour, can provide approximately 10 to 13% of the zinc, approximately17 to 22% of the copper, approximately 34 to 45% of the manganese, approximately 1% of the calcium, approximately 58 to 77% of the magnesium, approximately 8% of the potassium and approximately 39% of the phosphorus required for the RDA. Thus buckwheat noodles after cooking can provide a good potential source of zinc, copper, manganese, magnesium and phosphorus, but do not provide any significant amounts of calcium and potassium, for the RDA.

On the other hand, a considerable amounts of the seven minerals were subsequently found in the cooking water: approximately 18 to 38% of the minerals from the raw buckwheat noodles leached into the water upon cooking (Table 1). In Japan, there is a traditional dietary custom of drinking the hot cooking water left after cooking the buckwheat noodles, "soba-yu". It was believed that the cooking water may contain some required nutrients (Nagatomo, 1984), although the scientific basis for such a dietary custom had not been fully elucidated (Ikeda and Shimizu, 1993). The present finding shows that a considerable amount of the seven minerals evaluated in raw buckwheat noodles leached into the hot cooking water during cooking of the buckwheat noodles.

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

These findings suggest that the hot cooking water (soba-yu) after cooking of the noodles contain nutrients such as protein and some required minerals (Table 1). These findings (Table 1) may provide the basis for such a dietary custom.

The essential minerals released after in vitro enzymatic digestion of the buckwheat noodles and hot cooking water

Table 2 shows the amount (and the proportions to their original total content) of each essential mineral, i.e., zinc, copper, manganese, calcium, magnesium, potassium and phosphorus, released as soluble forms after amylase, pepsin plus pancreatin digestion of the buckwheat noodles and of the hot cooking water. A large variation in the proportions of the minerals released upon enzymatic digestion of the raw noodles was found among the seven minerals (Table 2): a higher proportion which was released after enzymatic digestion was found for zinc and potassium, whereas a lower proportion was found for calcium and manganese. Cooking increased the proportions of the

Table 1. Composition of seven essential minerals and total protein in buckwheat noodles and hot water soak[1)].

Foods examined	(A) Weight	Zn	Cu	Mn	Ca	Mg	K	P	Protein
	g	mg/EW[2)]							g/EW[2)]
Raw noodles	134	1.93	0.48	1.89	11.7	229	395	467	10.93
		(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)
Cooked noodles	239	1.14	0.31	1.34	8.4	184	163	276	6.63
		(59)	(65)	(71)	(72)	(80)	(41)	(59)	(61)
Hot water soak	311	0.66	0.15	0.37	3.0	45	151	85	2.35
		(34)	(31)	(20)	(26)	(20)	(38)	(18)	(22)

[1)] Values are means on a wet weight basis [n=4]. Values in parenthesis indicate per cent of each content of mineral and protein of the cooked noodles and the water soak to each content of the raw noodles when each content of mineral and protein in the raw noodles were taken as 100%.
[2)] EW, each weight indicated in (A) within the same line.

Table 2. Proportion to their original total contents of the amounts of seven essential minerals released as soluble forms after *in vitro* digestion of buckwheat noodles and hot water soak[1].

Foods examined	Zn	Cu	Mn	Ca	Mg	K	P
		μg/100 g food (%)				mg/100 g food (%)	
Raw noodles	1182±30	193±13	231±10	453±88	50.2±4.5	209.6±2.1	147.7±16.0
	(82.4)	(54.8)	(16.5)	(5.6)	(29.5)	(71.7)	(42.7)
Cooked noodles	337±22	94±19	151±21	774±32	32.7±4.4	53.0±3.8	52.9±1.6
	(70.2)	(72.3)	(26.9)	(22.2)	(42.8)	(77.6)	(46.2)
Hot water soak	177±8	40±4	86±13	293±39	12.1±1.0	39.2±4.2	23.9±1.4
	(83.0)	(85.3)	(73.3)	(30.4)	(84.1)	(80.6)	(87.4)

[1] Values are means±S.D. on a wet weight basis [n=4]. Values indicate means of proportion (%) of each mineral released after enzymatic digestion to each content of total minerals.

minerals released after the enzymatic digestion of noodles, except for zinc (Table 2). An increased proportion of the released amount after enzymatic digestion of the cooked noodles was especially found for copper, calcium, magnesium and manganese, when compared with that of raw noodles. On the other hand, high proportions of the minerals released by the enzymatic digestion of cooking water were found among six minerals, with the exception of calcium (Table 2).

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 much 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 enzymatic digestion method evaluating the availability of minerals with some foods, and the characteristics of their minerals (Ikeda, 1984, 1990; Ikeda et al., 1990; Ikeda and Murakami, 1995). The present study on cooked buckwheat noodles (Table 2) has shown that the enzymatic digestion enables large proportions of over 70% of three minerals, i.e., zinc, copper and potassium, to be released as soluble forms, but smaller proportions of two other minerals, i.e., calcium and manganese. On the other hand, there may be various, endogenous factors affecting the solubility of minerals upon digestion. Characterization of such factors will be an interesting subject in future.

In conclusion, the present study has shown that the content of seven minerals and protein in buckwheat noodles decreased on cooking (Table 1). About 20 to 40% of the mineral and protein content present in raw noodles were found in the hot cooking water (Table 1). High proportions of the minerals, released on the enzymatic digestion of the cooking water were found among six of the minerals with the exception of calcium (Table 2). These findings provide a scientific basis for the traditional dietary custom of drinking hot cooking water (soba-yu) left after cooking the buckwheat noodles. In spite of the reduced content of minerals and protein in the noodles upon cooking (Table 1), our evaluation suggested that one dish of cooked buckwheat noodles prepared from 100 g flour can be important sources of five of the minerals mentioned above, providing about 10 to 77% of the RDA for each mineral, with the exception of calcium and potassium. On the other hand, the present findings (Table 2) showed that enzymatic digestion of cooked noodles enables a large proportion of over 70% of three of the minerals, i.e., zinc, copper and potassium to be released as soluble forms. Such soluble minerals after digestion appear to be easily available for intestinal absorption, although the detailed mechanism involved remains uncertain. Research is currently in progress in our laboratory to characterize minerals in buckwheat and its products in view of their bioavailability.

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Food, chemical and nutraceutical research on buckwheat in Korea: Literature survey

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Key words: chronic diseases, flavonoids, functional food, Korea, nutraceuticals

ABSTRACT

Buckwheat *(Fagopyrum esculentum* Moench) is a grain that has been eaten in the form of noodles or buckwheat jelly in Korea for a long time. Since 1960, a successful campaign for increased rice production has, however, evoked a decrease in the value of buckwheat as a food. In these same decades, a significant increase in chronic diseases including cancer, diabetes mellitus, hypertension or arteriosclerosis have reminded us of the nutraceutical value of traditional foods such as buckwheat, because changes in food consumption patterns have regarded as one of the causes of the increasing incidence of these diseases. We will herein review nutritional topics about buckwheat, which were mentioned in literatures written mainly in Korean.

INTRODUCTION

In Korea, the first record about buckwheat was found in an old book "Hyangyakguguebbang (鄕藥救急方)" published in the era of the Koryo Dynasty (the 13th century). It shows that buckwheat was used to be make noodles at that time. Most of Korean now enjoys buckwheat as noodles, jelly or pancakes (Lee, 1992). Nevertheless, it is believed that buckwheat has been cultivated previously, as an alternative crop and buckwheat grain has been used as a hardy food in famine years. However, the production of buckwheat in Korea amounted to only 3,725 M/T in 2002. This implies that buckwheat in Korea has economical value as a minor or luxury food (NAQS, 2003).

During the past 30 years, the incidence of chronic diseases, including cancer, diabetes mellitus, hypertension or arteriosclerosis, have constantly increased while the dietary pattern has changed to a western style (Tchai, 1997; National Statistical Office, ROK, 2003). This has encouraged scientists to find possible nutraceutical function of traditional foods that we are now ignoring for their value in the diet.

We will herein review nutritional topics about buckwheat from reports published in Korean journals to elucidate the nutraceutical value of buckwheat grown in Korea. It is beneficial for global scientists to understand the accumulated scientific results or research situations on buckwheat in Korea.

VARIETIES

Buckwheat is an important food crop and the diversity of its genetic resources is centered in East Asia. Choi (1992) reported that his colleague collected 75 landraces of buckwheat from Korea and 146 landraces from overseas. The populations have been evaluated and developed in to new cultivars. Park et al. (2003) evaluated 220 landraces collected mainly from Kangwon Province, Korea. Currently, the recommended cultivars in Korea are as follows; Suwon 1, 2, 4, 12, 15 and 17, which were developed by selecting superior lines from the foreign cultivars. Although most of cultivars or landraces in Korea are classified as autumn-harvested types, Suwon 1 is classified as a summer-harvested type. In addition, we still cultivate a number of landraces as well as cultivars of Suwon 1 and 2 across the nation.

GENERAL CHEMICAL COMPONENTS OF SEED

General Composition

Kim et al. (1994a) reported the general composition of groats (dehulled seeds) for 9 cultivars. The content of crude protein, crude lipids and ash was in the range of 16.2 to 20.4%, 2.2 to 2.9% and 2.8 to 4.3%, respectively. Total carbohydrate was in the range of 63.1 to 68.1%. Shim et al. (1998) showed the composition of whole grains of 6 developed varieties and 7 landraces. The average content of crude protein, crude lipids and ash were 13.0±0.28%, 2.9±0.1 and 2.7±0.13%, respectively. The average content of nitrogen-free sugar was 70.2±0.4%. The differences in the content of carbohydrates shown in the literature was mainly due to the differences in the content of crude protein.

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Starch

Carbohydrates mainly consist of starch and dietary fiber. Kim et al. (1977) determined the physicochemical properties of starch prepared from buckwheat flour. Starch granules were in the range of 4.3 to 11.4 microns in size, the average being 7.8 microns, which shows a larger granule size than rice starch. The amylose content was 25%. Blue values (index of amylose and amylopectin ratio) for starch, amylose and amylopectin were 0.35, 1.38 and 0.24, respectively. The swelling power values below 60°C indicated a greater resistance toward swelling of buckwheat starch compared to wheat and rye starches. Also, amylograph results showed that the starch had an initial pasting temperature of 64.5°C, thereafter the viscosity increased sharply. Buckwheat starch had lower value for the time constant of starch gels at 21°C compared to that of wheat starch. Choo and Lee (1989) reported that granule size and shape of buckwheat starch was 3 to 10 microns and polygonal. Periodate oxidation test showed that amylose in the buckwheat starch had an average molecular weight of 133,690 degree of polymerization of 825, whereas amylopectin had a branch degree of 5.2 per 100 glucose unit of the main chain and 19.2 glucose unit per branch segment. Lee and Sohn (1992a), however, indicated that the data obtained from the periodate oxidation test may fluctuate greatly depending on the milling conditions of the buckwheat (Table 1). Lee et al. (2000a) reported that gamma irradiation treatment decreased the viscosity of buckwheat starch depending on the irradiation level: The decrease in the viscosity may reflect a progression in the depolymerization of starch.

Non starchy polysaccharides

The dietary fiber in cereals was determined using the Mongeau-Brassard method by Kim et al. (1993). The content of insoluble, soluble and total dietary fiber of buckwheat groats were 2.32, 3.79 and 6.11%, respectively on a dry matter basis. It is noteworthy to note that the content of soluble dietary fiber was higher than that of insoluble dietary fiber in the buckwheat groats. Lee et al. (1995b) analyzed indigestive and soluble polysaccharides of fractions, which were isolated from the supernatant and residue after enzyme treatment of raw, roasted and steamed buckwheat. Total ingestible polysaccharides (TIPs) content of raw, roasted and steamed buckwheat groats were 10.3, 10.0 and 14.7%, respectively. Thus, the steaming process may increase the content of ingestible polysaccharides in buckwheat groats. Gas chromatography showed that the TIPs consisted of glucose, xylose, arabinose, rhamnose, manose and galactose. The composition of dietary fiber of buckwheat grain during germination as reported by Lee et al. (1994c, 1995d) will be discussed later.

Protein and amino acids

Lee and Sohn (1992c) studied the electrophoretic pattern of protein extracted from buckwheat groats with 0.5 M NaCl. The relative proportions of the protein fractions were 21.1%, albumins; 13.8%, globulin; 28.4%, gliadin; 36.7%, glutelin. Choi and Sohn (1993) examined solubility, emulsion capacity and surface hydrophobicity of buckwheat protein isolate. The solubility and emulsion capacity had the lowest value at pH 4.5, the isoelectric point of the buckwheat protein isolate. They significantly increased as the pH was closer to higher acidic or alkali regions. The surface hydrophobicity showed the highest value at pH 2.0 and the lowest value at pH 11. Shim et al. (1998) determined the amino acid composition of whole buckwheat flour and showed that glutamate, arginine and asparagines were the major amino acids, whereas tryptophan, methionine and cysteine were minor ones.

Fatty acids

Lee and Sohn (1992b) analyzed the lipid component of buckwheat flour. Total lipids were extracted and fractionated to neutral lipids, glycolipids and phospholipids, respectively. Buckwheat lipids contained 82 to 96% neutral lipids, 2.0 to 11% glycolipids and 2 to 6% phospholipids. The neutral lipids were again separated into 6 fractions. Of these, triglycerides were dominant in the range of 89 to 92% and free sterol was in the range of 0.7%. Shim et al. (1998) analyzed fatty acid composition of total lipids extracted from whole buckwheat flour using the Folch method. Gas chromatographic analysis showed 18.8%, 16:0; 0.25%, 16:1; 2.3%, 18:0; 39.6%, 18:1; 29.1%, 18:2; 1.5%, 18:3 (n-3); 1.8%, 20:0; 3.6%, 20:1,

Table 1. Periodate oxidation results of buckwheat amyloses and amylopectins (Lee and Son, 1992).

Buckwheat	Amylose		Amylopectin	
	Molecular weight	Degree of polymerization	Degree of branching*	Glucose unit/Seg. of amylopectin
Whole	103,004	572	7.64	13.09
Dehulled	125,654	698	6.59	15.16

The data were obtained using the method of Gilbert and Spragg.
*Per 100 unit glucose of the main chain

where 16:1 etc. indicates fatty acid with 16 carbon atoms and one double bond etc.

Minerals

The mineral content of whole buckwheat flour was determined after nitric acid-sulfuric acid digestion by AAS. Whole buckwheat flour contained 6.9 to 16.5 mg/100 g of calcium, 5.2 to 50.4 mg/100 g of iron, 2.8 mg/100 g of zinc, 174 mg/100 g of magnesium, 14.3 mg/100 g of manganese and 426 mg/100 g of phosphorous. There were big variations in the iron content of buckwheat (Shim et al., 1998). Kim et al. (2000a) analyzed heavy metal content of cereals. Buckwheat flour contained 0.008 mg/kg of mercury, 0.17 mg/kg of lead, 0.037 mg/kg of cadmium, 0.12 mg/kg of arsenic and 1.88 mg/kg of copper.

Vitamins and minor components

The ascorbic acid content of whole buckwheat flour was measured with the dipyridyl method and was found to be in the range of 3.9 to 7.3 mg/100 g. On the other hand, total tocopherol content, as measured by HPLC, was in the range of 4.64 to 9.54 mg/100 g with a mean of 6.84 mg/100 g. γ-Tocopherol was the major form with a range of 4.32 to 8.58 mg/100 g in the tocopherol homologues. The β-form was present in lower amounts in buckwheat. Phytic acid may be classified as a good or bad component depending on its action. For instance, it exerts an anticarcinogenic or antioxidant action by chelating Fe ion but it also inhibits the absorption of minerals by chelating calcium or magnesium ion in the intestine. Phytic acid in whole buckwheat flour was determined using a colorimetric method. The content of phytic acid was in the range of 7.0 to 13.6 mg/g (average 10.3 mg/g) (Shim et al., 1998).

Rutin and its analogues

The rutin contents of buckwheat or its products have been determined by several investigators (Maeng et al., 1990; Kim et al., 1991; Kim et al., 1994b; Kwon, 1994; Park et al., 2000; Shim et al., 1998). Maeng et al. (1990) analyzed the rutin content in buckwheat and its products. The rutin contents in buckwheat groats ranged from 8.8 to 24.8 mg/100 g, depending on the species of buckwheat. Kim et al. (1994b) investigated the rutin content of 69 domestic and 43 introduced lines of buckwheat species by a spectrophotometric method. Shim et al. (1998) elucidated flavonoids profile of whole buckwheat flour using HPLC. They were composed of rutin, which was the major component in the range of 9.5 to 30.3 mg/100 g (average 17.4 mg/100 g), and quercitrin, myricetin and quercetin. Developed cultivars had higher rutin content than did the local landraces.

In Korea, buckwheat noodle (Makkuksoo) is traditionally made by an extrusion process of the buckwheat flour-water dough into boiling water and is then eaten immediately with seasonings. Kim et al. (1991) showed that the rutin in buckwheat noodles was considerably lessened depending on boiling time: Boiling for 5 minutes decreased rutin content to 50%. Park et al. (2000) determined the rutin content in products processed from groats, leave and flowers of buckwheat and showed that flower tea had the highest rutin content, ranging from 396 mg/100 g (first boiled tea) to 78 mg/100 g (second boiled tea).

Buckwheat germ

Lee et al. (1994a, c, 1995c, d) investigated the periodical changes in food chemical composition during buckwheat germination. Buckwheat grain was germinated at 10°C for 7 days and the content of dietary fiber was determined at 24 hours intervals using the AOAC method for total dietary fiber (TDF), and the Prosky method for insoluble (IDF) and soluble dietary fiber (SDF). The contents of TDF, IDF and SDF in germinated buckwheat seeds were 24.9%, 22.1% and 1.4%, respectively on a dry weight basis. Germination provoked an increase in TDF, SDF and IDF content with the increased degree of increase being the highest in SDF. Cellulose, hemicellulose and lignin were the main components of IDF. The ratio of cellulose, hemicellulose and lignin in neutral detergent fiber representing IDF was 54.7:6.5:38.7%. After 7 days of germination, amylase activity and glucose content increased 5.5 folds compared to dormant seeds. Calcium content increased until 4 days after germination and then decreased gradually. Kwon (1994) also showed a sharp increase in rutin content during buckwheat germination. The rutin content reached a level of 1.66 g/100 g in the buckwheat sprout 7 days after germination. There was no a dramatic change in the fatty acid composition of buckwheat during germination.

IN VITRO BIOLOGICAL FUNCTIONS OF BUCKWHEAT

Lee et al. (1996b) showed that crude hemicellulose or alcohol-insoluble hemicellulose isolated from buckwheat groats inhibited the activities of α-amylase, lipase, trypsin and chymotrypsin. Roasting or steam pretreatment of buckwheat groat prior to isolation did not show any evident affect on the enzyme activities. However, the digestibility of casein by trypsin was significantly inhibited when low molecular weight insoluble polysaccharides isolated from roasted buckwheat groat were added (Son et al., 1995). Lee and Shin (1998) determined *in vitro* starch hydrolysis rate of cereals using α-amylase. The hydrolysis rate of the starch by α-amylase for 2 hours was faster than for rice. However, when the rate was expressed as an incremental area, no significant difference was found between buckwheat and rice.

Ha et al. (1998) studied the *in vitro* inhibitory activity of buckwheat extract against hydroxy-methylglutaryl-CoA (HMG-CoA) reductase. An inhibitory activity was shown when the methanol extract (26.2% decrease) was added to the assay, but not in a 70% ethanol-water extract (Table 2). Lee et al. (1991b) also showed an *in vitro* inhibition action in an extract from buckwheat hulls.

A buckwheat methanol extract inhibited the *in vitro* activities of lens aldose reductase and α-amylase. The extent of inhibition was dependent on the cultivar. In addition, buckwheat protein suppressed *in vitro* angiotensin I, converting enzyme (ACE) activity. Protein (precipitated at pH 6 buffer) especially had an equivalent suppressive effects on rutin. Quercetin exerted a 10 times stronger inhibitory action than rutin against ACE activity. Protein extracted from buckwheat groats had higher hydrophobicity than casein, which is related to its sterol binding capacity (Choi et al., 2000).

The antioxidant activity of buckwheat is likely to depend largely on the cultivar. Choi et al. (2000) showed that an extract from Suwon 4 cultivar exerted an equivalent antioxidant effect to rutin. The antioxidant activities of buckwheat seeds or vegetables have been reported by several researchers (Kim, 1996, Suh et al., 1997; Choi et al., 1998; Choi et al., 2000).

On the other hand, Choi et al. (1998) investigated the anticarcinogenic activity of 70% ethanol extract from buckwheat groats. The conventional short-term antipromoter assay system using activation of the Epstein Barr Virus (EBV) suggested that buckwheat extract has a higher inhibitory effect when compared to rice or brown rice on the promotion of cellular carcinogenesis. Ham et al. (1994) indicated that an ethanol extract from buckwheat vegetables exerted antimutagenicity under conditions of low concentration. But it did comutagenicity under conditions of high concentrations in a spore rec- assay using *B. subtillus* H17 (rec+) and M45 (rec−). In a Ames test, the ethanol extract reduced the mutagenicity of MNNG, B(a)P, 2AF or Trp-P-1 in *S. typhimurium* TA98 and TA100.

Table 2. The inhibitory effect of 80% methanol extracts from cereals and legumes on microsomal HMG-CoA reductase activity* (Ha et al., 1998).

Samples	Specific activity (nmole/mg protein/min)	Inhibition rate (%)
Control	4.15	
Sorghum	2.29	44.7
Prosomillet	1.13	72.7
Barley	4.45	−7.3
Naked barley	3.37	18.8
Glutinous millet	4.66	−12.2
Buckwheat	3.06	26.2
Black rice	5.70	−37.4
Red rice	4.76	−14.7
Brown rice	4.15	0.0
Job's tear	3.93	5.2
Black sesame	3.89	6.2
Mungbean	4.02	3.0
Small red bean	5.69	−37.3
Corn	4.40	−6.1

*The liver microsomal enzyme source was taken from a rat fed with a diet containing 0.4% cholestyramine for 10 days.

IN VIVO NUTRITIONAL BIOCHEMICAL FUNCTIONS OF BUCKWHEAT

Growth

Lee (1972) compared the weight gain in rats fed with a rice diet or a rice diet containing buckwheat (20.0%) for 21 days, and showed that the group given a mixture of rice and buckwheat increased their weight more efficiently than the group given only rice. The addition of buckwheat to rice may improve the protein score in the diet.

Blood glucose and lipids: Animal studies

Lee et al. (1994b) evaluated the effects of 50% buckwheat groats (raw, roasted or steamed) diet on growth parameters, plasma glucose and lipids in streptozotocin (STZ)-induced diabetic rats for 14 days. There were no differences in growth parameters among the groups. The buckwheat diet decreased the fasting plasma glucose level by 18–37% as compared to the diabetic control group (547±29 mg/dl). The lowering degree of the plasma glucose level depended on the type of buckwheat: the roasted buckwheat diet induced the lowest blood glucose level, and raw buckwheat was intermediate. Also, the buckwheat diet reduced the concentration of blood triacylglycerol in similar pattern to the blood glucose concentration. In an another report, they (1996a) showed that a buckwheat diet induced an increase in pancreas weight, especially in the raw buckwheat-fed group. Choi and Lee (1997) studied the effects of exercise on the response of blood glucose and cholesterol level in streptozotocin-induced rats fed with buckwheat for 4 weeks. They found that the beneficial effects of buckwheat in lowering blood glucose were found only when the diabetic rats was exercised. The total plasma cholesterol level was not affected by either diet or exercise. Choe et al. (1991) determined the response of blood glucose from oral glucose tolerance test using normal rats fed with buckwheat flour for 4 weeks. The glucose tolerance curve showed clearly the low blood glucose response pattern in rats fed with buckwheat flour as compared with the control group. Mobilization of serum insulin was 1.5 times faster in the buckwheat group than in the control group.

Choi et al. (1992) reported that a diet of 50% buckwheat noodles consisting of 30% buckwheat and 70% wheat flour did not alter the serum and liver cholesterol levels in normal rats, but it showed a trend to decrease the concentration of serum and liver triacylglycerol when compared to a diet containing 100% wheat flour. In contrast to buckwheat flour, Choi et al. (1994) suggested a hypocholesterolemic effects of dried buckwheat vegetables harvested in the range of 10–15 cm in height on the lipid parameters in rats for 4 weeks. Groups fed with the dried buckwheat vegetables (5.0% of diet) had a lower serum cholesterol level as compared with the control group. The lowering effect of dietary rutin was similar to that of the dried buckwheat vegetables. Dietary rutin efficiently decreased serum triacylglycerol levels (Table 3).

Blood glucose and lipids: human studies

Lee and Shin (1998) measured the levels of blood glucose over 2 hours periods after feeding healthy volunteers 50 g carbohydrate portions. The glycemic index values to rice (100%) used as the standard were 122±2% for glutinous sorghum, 90±12% for unpolished rice, 79±5% for buckwheat groats and 63±6% for barley. Lee et al. (1995a) studied the effects of a buckwheat diet on serum glucose and lipid level in 9 NIDDM (noninsulin dependent diabetes mellitus) volunteers (female, age 46.9±2.7). They were fed rice for 1 week and then rice-buckwheat (1:1) was eaten for 2 weeks. There were no differences in intake of energy, carbohydrate, protein and fat between the two experimental periods. The mean total glycohaemoglobin, frutosamine, and total cholesterol levels at the end of buckwheat diet was significantly lower than at the end of the control diet. Fasting serum glucose and triacylglycerol level were not changed. Kang et al. (2001) compared the dietary effects of buckwheat versus rice on postprandial blood glucose and insulin levels in 11 normal subjects. Polished rice or buckwheat groats containing 50 g as carbohydrates was offered after an overnight fast. Postprandial capillary glucose and serum glucose responses to buckwheat 30 to 90 min after feeding were significantly lower than those to rice. Serum insulin response to buckwheat depicted a similar pattern to serum glucose level (Fig. 1).

Blood Pressure

Lee et al. (2000b) investigated the dietary effects of germinated buckwheat seeds in spontaneous hypertensive rats (SHR) for 6 weeks. Buckwheat seeds were germinated at 10°C for 5 days and then dried. Dietary germi-

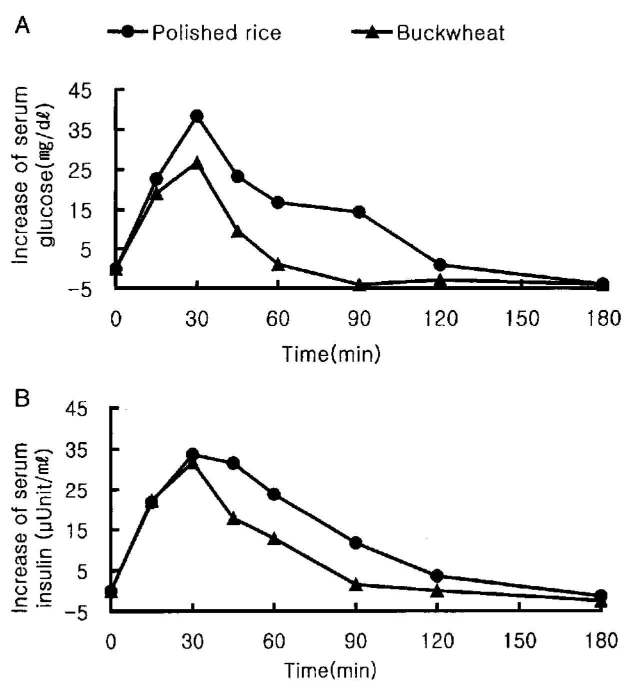

Fig. 1. Effects of dietary buckwheat on blood glucose and insulin levels in healthy subjects. (A) The mean incremental serum glucose responses and (B) The mean incremental serum insulin responses (Kang et al., 2001). 11 healthy subjects of mean age 23.3±2.2 years ingested cooked rice or buckwheat after an overnight fast. Blood was periodically collected before, and from 15 to 180 min after test meals.

Table 3. Concentration of lipids in liver and serum of rats fed with buckwheat vegetables (Choi et al., 1994).

	Liver		Serum	
	Cholesterol	Triacylglycerol	Cholesterol	Triacylglycerol
Control	6.3±0.61[ab]	22.4±3.2	93.2±7.7	185±26
Vegetables[1]	4.8±0.60[a]	22.5±3.9	79.4±4.7	159±19
Rutin[2]	6.8±0.44[b]	21.6±1.4	79.6±6.0	104± 6

Mean±S.E. of 7 rats

Rats were fed with each purified diet for 4 weeks.

[a,b] Significantly different at P<0.05

[1] Vegetable diet contained 3.5 g rutin/kg of diet

[2] Rutin diet contained 2.5 g rutin/kg of diet

nated buckwheat decreased systolic blood pressure more evidently in male rats than in female rats. Choi et al. (2001) evaluated the hypotensive effects of buckwheat groats as compared to rice in SHR. The rats were fed with a purified diet containing 60% buckwheat powder or rice powder. Blood pressure was monitored for 6 weeks. Dietary buckwheat significantly decreased the systolic blood pressure (Fig. 2).

Allergy

Lee et al. (1991a) evaluated the allergenic antigenicity of buckwheat flour in Korean atopic children. They isolated and partially characterized the buckwheat flour allergens. Electroblotting analysis with SDS–PAGE revealed more than 30 allergenic components of molecular weights between 65 kd and 12.5 kd being bind specific to IgE. Among these allergens, seven components of 43, 41, 37, 34.5, 32.5, 22 and 12.5 kd bound commonly the sera of the 13 atopic children tested. Chae et al. (2001) reported three cases of buckwheat allergy. The three cases showed respiratory discomforts after exposure to buckwheat and revealed positive reactions to buckwheat in the skin prick test. Lee and Hong (1995) reported two cases of children nocturnal asthma induced by pillows filled with buckwheat hulls. Interestingly, Kim et al. (2003) suggested an anti-allergic effect of buckwheat grain methanol extract (BGME) acting on inhibition of histamine release and cytokine gene expression in mast cells. They also showed that the oral, intraperitoneal and intradermal administration of BGME to male rats significantly inhibited the compound 48/80-induced vascular permeability documented by Evans blue extravasation.

FOOD PROCESSING AND STORAGE

Chung and Kim (1998a, b) studied the rheological properties of dough made from composite flour containing 30% buckwheat flour and 70% wheat flour with the addition of vital wheat gluten and/or gums. The addition of gluten increased the extension and strength of the dough, but decreased the resistance to extension. Gum guaiac and xanthan gum added at the 0.2% level had little effect on extensograph data of the dough. The addition of vital wheat gluten improved the properties of buckwheat bread dough better than water-soluble gums. Synergistic effects on bread quality were observed when the vital wheat gluten and xanthan gum or gum guaiac was used together. On the other hand, Kim et al. (2000b) showed that bread made with 10% buckwheat-90% wheat flour was much better when compared to bread containing higher ratios of buckwheat flour in sensory evaluations. Lee and No (2001) showed that major bacteria causing spoilage of buckwheat starch jelly was *Serratia liquefaciens* and *Staphylococcus lentus.* They also indicated that the addition of 1.0% chitosan could expand shelf life by 1–2 days. Yoon et al. (1988) observed a periodical change in the storage quality of acacia and buckwheat honey at various temperatures for 1 year, and indicated that buckwheat honey had less change in food chemical parameters than honey originated from the acacia flower. Buckwheat sprouts were developed and utilized in the form of fresh or seasoned vegetable (Kim et al., 2001). They found that buckwheat sprouts provide an abundance of nutritional factors such as protein, essential amino acids, minerals, crude fiber and rutin.

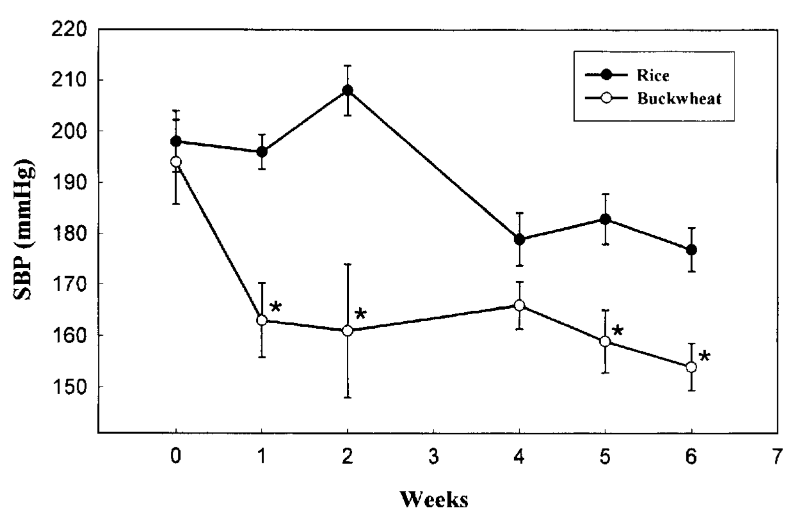

Fig. 2. Effects of dietary buckwheat on systolic blood pressure of spontaneous hypertensive rats (Choi et al., 2001). 20 weeks old Rats (male SHR/NCjr) were fed a diet containing 60% buckwheat or rice for 6 weeks. Blood pressure was determined every 7 days in all animals using the standard tail-cuff method (Le 5007, Letica Co., Spain). *P<0.05, compared to the rice-fed group.

EPILOGUE

In Korea, research on buckwheat is still at a fundamental level. Rice has been kept as a symbol of plenty, whereas buckwheat has been recognized as symbol of poverty in Korea. Anyway, financial support or ardor for buckwheat science has been really limited compared to other cereals. In 2001, the 8th International symposium on buckwheat at Chunchon, Korea has brought new significance on buckwheat research in Korea. It is believed that progressive growth in the buckwheat industry should also lead to encouraging researchers to seek substantial value or the discovery of new functions in buckwheat or its related products for improving health, even though most of the industry is still a house scale business. For instance, we are trying to find new resources as nutraceuticals from buckwheat plants, especially for aged peoples suffering from chronic diseases. In this context, many components of buckwheat can be strong candidates as functional resources. New plant biotechniques in Korea also hold promise for us to approach to the buckwheat development and research as related to new biological functions as well as to high-yielding varieties.

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A preliminary study on the herbicidal weed control in buckwheat

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ABSTRACT

Weeds pose a major threat to the productivity of buckwheat in the dry temperate regions of India. The major weeds include *Digitaria sanguinalis* and *Equisetum arvensis.* To timely manage this problem, judicious use of herbicides could be an alternative to manual weeding, which is tedious as well as cumbersome. Feld experiments were conducted during the summer seasons of 1999 to 2001 at Sangla to evaluate some effective herbicides against weeds in Tartary (phafra) and common (ogla) buckwheat. In Tartary buckwheat, alachlor (1–1.5 kg/ha) and in common buckwheat both alachlor (1 kg/ha) and metalochlor (1 kg/ha) were found to be best in suppressing the weeds and thereby improving productivity to a larger extent. Among the herbicides tested atrazine (1 kg/ha), isoproteuron (1 kg/ha) and pendimethalin (1.5 kg/ha) were found to be highly phytotoxic while fluchloralin (1 kg/ha) and pendimethalin (1 kg/ha) were less toxic. Butachlor, one of the selective herbicide, failed to control weeds at 1 kg/ha. Seed yield of common and Tartary buckwheat were positively correlated with straw yield and other yield contributing characters but negatively correlated with weed biomass.

INTRODUCTION

Buckwheat (*Fagopyrum tataricum* Gaertn and *Fagopyrum esculentum* Moench) occupies about 70% of the cultivated lands in the higher Himalayas and are grown either as a solid stand or under apple orchards. It is one of the most important and nutritious crops of the mountain regions and is the only crop which can be grown up to 4500 m (Joshi and Paroda, 1991). Buckwheat is a staple food in the high mountains, at the lower elevations it is also grown for its tender leaves which are consumed as human food. It is a short duration crop and fits well in the higher mountain regions where the growing season is of a limited period due to early winters (Joshi, 1999). Under ideal conditions common buckwheat emerges within 3–4 days whereas Tartary buckwheat takes 6–7 days. The crop grows rapidly and covers the ground very quickly. The buckwheat crops that produce a good canopy are very good competitors for weeds and generally smother them out (Hore and Rathi, 2002). However, if weeds are more advanced than the crop the buckwheat is severely affected. Therefore, all weeds, including volunteer plants must be controlled (Parodi and Nebreda, 1998). In buckwheat a higher seeding rate is generally used to promote faster canopy development and a higher population for better weed management (Hore and Rathi, 2002; Chernatskii, 1975). However, one weeding and hoeing at 20–25 days after seeding (DAS) or even later is imperative to sustain crop productivity. But manual weeding is tedious and cumbersome as well as being costly. Moreover, unavailability of labour at the right time warrants some alternatives, such as herbicidal weed control. The present study was conducted with the objective to develop selective herbicidal weed control to reduce women drudgery.

MATERIALS AND METHODS

Field experiments were conducted in three consecutive summers (1999–2001) at Sangla (2591 m above msl, 31°25'56"N latitude and 78°15'4"E longitude) in a randomized block design with three replications. The soil of the experimental plot was sandy loam, neutral in reaction (pH 6.8–6.9), low in available N (215 kg/ha), medium in P (16.4 kg/ha) and K (118 kg/ha) with an organic carbon content of 0.40–0.48%. Twelve weed control treatments in Tartary buckwheat (Table 1) and nine in common buckwheat (Table 2) were selected for the study. In the first year, six herbicides (each at 1 kg/ha) were compared with the existing farming practice of hand weeding in Tartary buckwheat. In the next year, a weedy check plus two more herbicides *viz.* pretilachlor (1 kg/ha) and oxyfluorfen (0.25 kg/ha) were added, while a higher rate (1.5 kg/ha) of pendimethalin as well as alachlor were also tested. However, in the third year butachlor, isoproteuron, atrazine and pendimethalin (at 1.5 kg/ha) were excluded from the treatment schedules because of their toxicity and/or poor efficacy. In common buckwheat, the experiment was carried out for two years (1999 and 2000) and the treatment schedule for both the year were the same except for the weedy check which was not included in the first year. The Tartary buckwheat was sown in the first week of June and common buckwheat in the third week of June in respective years. Seeds of improved genotypes of Tartary buckwheat (Sangla B-1) and common buckwheat (OC-2) were sown at 40 kg/ha in lines 30 cm apart. One half of the N (40 kg/ha) and all of the P_2O_5 (40 kg/ha) and K_2O (20 kg/ha) was applied as a basal treatment. The remaining N was top dressed at the knee height stage of the crops. Fluchloralin was applied before sowing and incorporated into the upper 2–3 cm layer of the soil. The rest herbicides were applied one day after sowing. Other crop management practices were in accordance with the package of practices for these crops (HPKV, 1993). Data on weed dry weight were recorded at maturity. Seed and straw yields were harvested from each plot.

RESULTS AND DISCUSSION

The major weed species which occurred in Tartary and common buckwheat was *Digitaria sanguinalis* L. Scoop (81.5, 95.4 and 73.6%, respectively during 1999, 2000 and 2001 in Tartary buckwheat and 85.6 and 87.2%, respectively in common buckwheat during 1999 and 2000) and *Equisetum arvensis* L. In addition, *Malva verticillata* and *M. rotundifolia*, *Chenopodium album*, *C. murale* and *Amaranthus* spp. are known to be associated with the crop but their populations were extremely low. During 1999, *Setaria viridis* was also found in the common buckwheat plots.

Tartary buckwheat

Analysis of the data (Table 1) revealed that the pre-emergence application of alachlor at 1.5 kg/ha was the most effective herbicidal treatment in controlling weeds and increasing seed and straw yields. The increase in seed yield due to alachlor 1.50 kg/ha over hand weeding was 57.85% and 20.54%, respectively and over weedy check was 169.5% and 89.63%, respectively, during 2000 and 2001. Other effective herbicidal treatments were alachlor and pretilachlor each at 1 kg/ha and oxyfluorfen at 0.25 kg/ha. However, pendimethalin at 1 kg/ha and fluchloralin at 1 kg/ha were slightly toxic, although the toxicity in the case of the latter was overcome as indicated by the yield attributes and yields, which were not statistically different from those obtained under alachlor (1 kg/ha), pretilachlor (1 kg/ha) and oxyfluorfen (0.25 kg/ha). *Digitaria* was the most dominant weed and its sensitivity (susceptibility) towards oxyfluorfen (Rao, 1983), alachlor, pendimethalin, fluchloralin (Rana et al., 1999) has been reported in other crops.

Isoproturon and atrazine, each at 1 kg/ha, and pendimethalin at 1.5 kg/ha were extremely phytotoxic to Tartary buckwheat. They caused acute toxicity symptoms when the crop was 15–20 cm in height. The healthy plants suddenly start withering and dying. The long time period between application and inception of toxicity symptom in the crop (i.e., 15–20 days) may presumably be due to a intermediate process of metabolic degradation of the compound (parent molecule) in the plant system before the respective herbicide or intermediate (reverse metabolism) compound was converted into the real toxic

Table 1. Effect of herbicide treatments on weeds and Tartary buckwheat.

Treatment	Dose kg/ha	Weed dry weight ($g\ m^{-2}$)			Seed yield ($t\ ha^{-1}$)			Straw yield ($t\ ha^{-1}$)			Seeds plant^{-1}			Plant density*
		1999	2000	2001	1999	2000	2001	1999	2000	2001	1999	2000	2001	2000
Weedy		—	571.6	263.4	—	0.82	1.64	—	1.24	2.15	—	131	165	271
Hand weeding		41.7	153.9	22.4	1.20	1.40	2.58	3.04	1.74	3.12	283	173	245	278
Butachlor	1.00	49.0	504.3	—	1.50	1.00	—	3.26	1.38	—	307	136	—	297
Isoproturon	1.00	55.3	564.8	—	1.49	0.14	—	2.99	0.14	—	309	129	—	29
Atrazine	1.00	65.7	508.2	—	1.30	0.13	—	2.72	0.12	—	281	132	—	14
Pretilachlor	1.00	—	180.8	125.8	—	1.62	1.99	—	2.11	2.30	—	189	201	301
Alachlor	1.00	26.7	120.0	30.9	1.62	1.71	2.90	3.60	2.44	3.52	325	199	278	287
Pendimethalin	1.00	26.3	235.5	90.4	1.61	1.04	2.41	3.49	1.11	3.01	324	172	240	166
Alachlor	1.50	—	22.2	15.8	—	2.21	3.11	—	3.14	3.89	—	219	301	303
Pendimethalin	1.50	—	157.1	—	—	0.22	—	—	0.29	—	—	176	—	44
Fluchloralin	1.00	44.7	106.8	40.7	1.38	1.79	2.79	2.79	1.99	3.39	291	203	257	240
Oxyfluorfen	0.25	—	186.9	139.8	—	1.58	1.33	—	2.03	1.66	—	193	196	265
LSD (P=0.05)		20.2	75.2	17.2	0.17	0.18	0.63	0.31	0.32	0.31	18	16	19	41

* Number of plants m^{-2}

factor. Phytotoxicity due to atrazine was noticed earlier than that for isoproturon or pendimethalin. This may be explained by the fact that the active toxic moiety in atrazine gets metabolically activated earlier in plant system and thus the toxicity are expressed at an early stage of crop growth. Though atrazine and pendimethalin are used primarily as pre-emergence treatments, their post emergence activity through shoot absorption has also been established (Gupta, 1993). Isoproturon is recommended to be applied both as pre as well as post emergence, thus direct inhibition by means of shoot absorption of the parent molecule in Tartary buckwheat may also occur. Butachlor has become the most important herbicide in rice crops (Gupta, 1993), at 1 kg/ha. Although it was selective, it failed to check the weed growth. Atrazine (Rao, 1983) and isoproturon also were not effective against weeds. The straw yield assumed a somewhat similar trend as to that of seed yield. The increased straw yields could be of great significance in respect to sustainable growth of animal husbandry, particularly in the dry temperate regions, where scarcity of fodder is of common occurrence due to short growing seasons.

In the present study seed yield was negatively correlated to weed biomass [r, –0.57, –0.73** (1% level of significance) and –0.83* (5% level of significance) during 1999, 2000 and 2001, respectively] and positively correlated with straw yield (r. 0.78*, 0.98** and 0.99**) and number of seeds/plant (r, 0.97**, 0.77** and 0.93**) indicating high degree of association which was affected by the presence of weeds.

Common buckwheat

Alachlor (Fig. 1) and metolachlor, each at 1 kg/ha, reduced weed biomass and increased yield contributing traits and yield by 135.8% and 132.3%, respectively over the weedy check (0.37 t/ha). The increase in yield due to alachlor and metolachlor over manual weeding (0.76 t/ha) was 12.2% and 7.77%, respectively. Pretilachlor at 1 kg/ha was the next best herbicide during 2000. Likewise as in Tartary buckwheat atrazine, isoproturon and

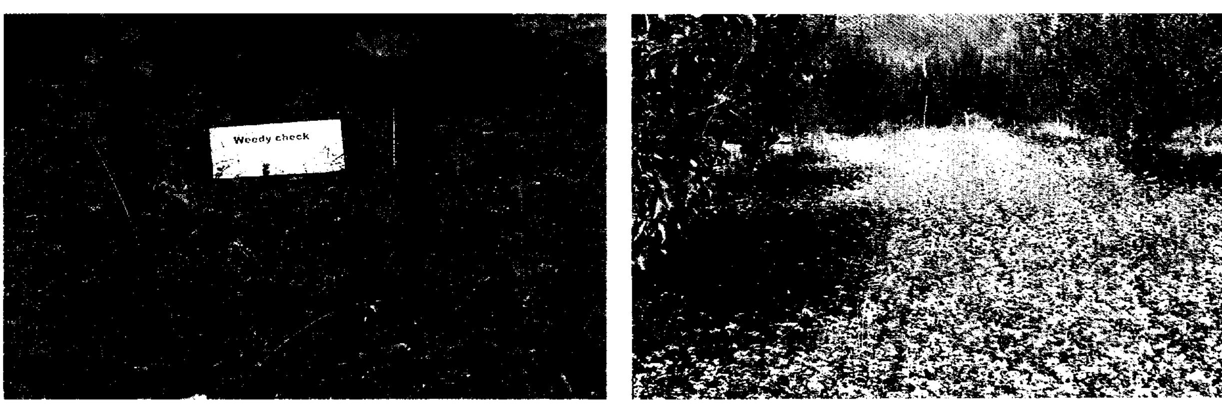

Fig. 1. Common buckwheat crops as influenced by alachlor treatment (right) as compared to weedy check (left).

Table 2. Effect of herbicide treatment on weeds and common buckwheat.

Treatment	Dose kg/ha	Weed dry weight (g m^{-2})		Seed yield (t ha^{-1})		Straw yield (t ha^{-1})		Seeds $plant^{-1}$		Plant height (cm)		Plant density*	
		1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
Weedy	—	—	307.3	—	0.37	—	1.09	—	42.8	—	61.7	—	232
Hand weeding	—	20.6	50.0	0.77	0.75	2.57	2.17	65.6	78.0	101.7	90.5	217	244
Butachlor	1.00	70.4	285.4	0.59	0.41	2.13	1.21	57.1	49.1	95.7	68.1	196	229
Isoproturon	1.00	82.6	288.1	0.56	0.05	2.11	0.16	57.0	50.1	92.0	65.9	208	25
Atrazine	1.00	80.8	262.9	0.25	0.02	0.47	0.07	50.9	48.3	79.0	63.8	89	12
Pretilachlor	1.00	40.7	61.9	0.66	0.64	2.41	1.86	63.4	72.2	103.7	95.7	202	243
Alachlor	1.00	18.4	17.0	0.76	0.87	2.96	2.60	69.9	96.5	111.7	100.3	215	241
Pendimethalin	1.00	35.8	58.9	0.69	0.11	2.62	2.37	64.5	64.6	109.7	82.0	177	67
Metolachlor	1.00	32.0	18.7	0.71	0.86	2.69	2.62	64.7	91.5	107.7	101.0	217	232
LSD (P=0.05)		16.3	23.8	0.06	0.08	0.21	0.25	5.3	12.0	6.4	9.7	22	23

* Number of plants m^{-2}

pendimethalin, each at 1 kg/ha, were phytotoxic. Isoproturon, atrazine and butachlor were not effective against the weeds (Table 2). Common buckwheat seed yield was negatively correlated with weed biomass (r, –0.80* and –0.69* during 1999 and 2000, respectively) and positively correlated to straw yield (r, 0.99** and 0.77*), seeds per plant (r, 0.93** and 0.83**), plant height (r, 0.95** and 0.83**) and final plant stand at harvest (r, 0.90** and 0.87**) showing positive influence of weed control in common buckwheat.

CONCLUSION

The findings of this investigation suggest an application of alachlor 1–1.5 kg/ha in Tartary buckwheat and alachlor and metolachlor at 1 kg/ha in common buckwheat for sustainable crop production through effective weed control. Both species of buckwheat did not vary much in their response to herbicides tested. Since continuous use of same herbicide year after year may create some serious developments, such as development of resistant biotypes, rotational use of the available herbicides including pretilachlor, fluchloralin or oxyfluorfen is recommended. Integrated weed management involving herbicides and cultural practices will be our future line of work.

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Effect of buckwheat sprout intake on population increase of *Caloglyphus berlesei* (Michael) (*Acari: Acaridae*)

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ABSTRACT

Young buckwheat sprouts were served to a free living acarid mite species, *Caloglyphus berlesei* (Michael), which were kept under constant conditions of ca. 20°C, and 95–100% humidity. The most important live parameters (average) obtained in this study were as follows: life history was 17.7 days (without hypopus instar), eclosion of imagines was 86.0%, longevity of adults was 23.8 days, fecundity of females was 237.4 eggs per lifespan. These data were compared with those of the same species fed on beebread and with similar data from *Rhizoglyphus echinopus* (Fumouze et Robin) fed on buckwheat sprouts. These comparisons showed that buckwheat sprouts were a good nourishment source for *C. berlesei*. Buckwheat sprouts appear to be worthy of recommendation as an effective medium for laboratory mite culture and production of acarological material for experimental and educational purposes.

INTRODUCTION

Acarid mites, *Caloglyphus berlesei* (Michael) belong to a free living mite species. The mites in soil, feeding on decaying plant materials, e.g. fruits, bulb, root or rhizome vegetables and ornamental plants, potatoes, and mushrooms. They often occur in compost, green- and mushroom-houses and field cultivation, in damp stored products, e.g. grain, various plant seeds and food-stuffs (Hughes, 1976; Turk and Turk, 1957; Zakhvatkin, 1941). *Caloglyphus berlesei* was also observed as invader of broiler-houses, colonizing poultry manure and as necrophagous species infesting bodies of dead birds, e.g. chickens, geese. *Caloglyphus berlesei* also utilizes other kinds of animal food; sometimes this species will feed on dead insects, e.g. grubs of scarabeid beetles (Lipa and Chmielewski, 1966; Chmielewski and Lipa, 1967). Its association with other arthropods, mostly beetles and other insects, is mainly phoretique in nature. I have observed *C. berlesei* feeding on nematode infested rooting plants, e.g. carrot, onion, tomatoes and other vegetables too. Hypopodes of *Caloglyphus* were sometimes found under the wings (elytrae) and attached to the surface of abdomen segments of some insect species belonging to the family *Scarabeidae* or on the body surface of larvae, pupae and imagines of these beetles, e.g. *Amphimallon solstitialis* L., *Melolontha melolontha* L. and *Phyllopertha horticola* L. *Caloglyphus berlesei* was also oberved as phoretant attached to house fly (*Musca domestica* L.) and other common *Diptera*.

The biochemical composition and utilization of buckwheat sprouts as a valuable, functional vegetable has been reported (Kim et al., 2001). So buckwheat sprouts were considered to be an attractive nourishment for some plant feeding mites. The purpose of this study was to test buckwheat sprout intake as a feed for *C. berlesei*.

MATERIAL AND METHODS

The acarological materials were living mites of *C. berlesei* collected from decaying plant materials, e.g. compost for mushroom-houses. These mites were used as initial specimens for monocultures that were grown under constant conditions of ca. 20°C and 95–100% humidity. Whole-meal and white bread were used as feed at the beginning for rearing the mites, but later, the stock cultures were kept and biological experiments were conducted on buckwheat sprouts that were grown for 4–7 days in Petri dishes.

The methods adopted in this study were similar to those used in the study on bulb mites, *Rhizoglyphus echinopus* (Fumouze et Robin.) (Chmielewski, 2001). Fecundity and longevity of the mites were examined as follows. Resting nymphs were isolated from mass cultures, just before their transformation into imagines, and put into separate Petri dishes and rearing cages containing food (buckwheat sprouts). After eclosion of imagines they were paired and mated; 25 pairs were formed and each pair (1♀+1♂) was placed into a separate rearing cage and supplied with pieces of plant food. Longevity was recorded and deposited eggs were counted every 2–3 days. The number of newly laid eggs was noted down every 2–3 days and the eggs were then removed from the rearing cages; fresh food was added if necessary.

The observations of the developmental cycle were based on 100 one-day-old eggs (10 rearing cages × 10 freshly laid eggs), which were placed into rearing cages on pieces of young buckwheat plants. Advances of ontogenesis and natural mortality of juvenile instars during their development were recorded every 1–2 days; after eclosion of the adults they were counted and removed; sex of imagines (frequency of males and females) was recorded. Nourishment was changed or added according to the need.

RESULTS AND DISCUSSION

The life parameters of *C. berlesei* fed buckwheat sprouts show that buckwheat sprout is a good nourshipment source of food for this species (Table 1). In ontogenesis of *C. berlesei*, heteromorphic forms, commonly known as heteromorphic deutonymphs, or so called hypopi (or hypopodes), were found between homomorphic nymphs i.e. proto- and deuto- nymphs, in addition to the homomorphic stages (egg, larva, protonymph, deutonymph, imago (♀ or ♂). This phenomenon is typical for the *Acaridae* family. The percentage of heteromorphs ranged from several to over 25% of the total number of freshly emerged males.

Hypopus specimens are especially adapted for the spreading and survival of some acaroid and anoetoid (*Acaroidea, Anoetoidea*) mite species under unfavourable life conditions which occur in nature (Behura, 1957; Chmielewski, 1971a, 1977; Hughes, 1976; Kuo and Nesbitt, 1970; Wallace, 1960). Transmitters or transporters of *C. berlesei* hypopodes are mainly the beetles which usually live in the same habitations. A list of some phoretic mite species and their hosts is given in acarological literature (e.g. Hughes, 1976; Turk and Turk, 1957; Zakhvatkin, 1941).

The importance of the heteromorphic males is not exactly known; this character may play a part in fighting with homomorphic males for females (sex rivalry); the thick and strong third pair legs of pleomorphic males may favor them when competing for females with other males and also help them to attach to females during copulation. There are reports in literature and remarks on andropolymorphism of this and other acarid mite species, e.g. *Caloglyphus michaeli* (Oudemans), *Rhizoglyphus echinopus* (Fumouze et Robin), *Lardoglyphus konoi* (Sasa et Asanuma) (Chmielewski and Lipa, 1967; Chmielewski, 1971b, 1983; Radwan, 1993a, b, 1995, 2000; Timms et al., 1980, 1980, 1981; Woodring, 1969a, b).

Another interesting biological feature of this and other species of the genus *Caloglyphus* is an aparity phenomenon, i.e. the development of progeny (various stages—eggs, larvae, nymphs, including hypopi, except imagines) inside the body of dead females (as opposed to normal development outside the female body i.e. viviparity); in this case the hatching larvae and other developing juvenile specimens use the internal mother's tissues as food (Lipa and Chmielewski, 1966; Chmielewski and Lipa, 1967). Aparity has been reported long ago in other species which are representatives of other mite groups, e.g. in *Oribatida* (Vitzthum, 1943).

The life history of *C. berlesei* fed buckwheat sprouts at room temperature (ca. 20°C) and high relative humidity (95–100%) usually lasts about 2–3 weeks (without hypo-

Table 1. Bionomics of *Caloglyphus berlesei* (Michael) and *Rhizoglyphus echinopus* (Fumouze et Robin) fed buckwheat sprouts or bee-bread.

Life parameters	Media and mite species (reference)		
	Buckwheat, *C. berlesei* (present results)	Bee-bread, *C. berlesei* (Chmielewski, 2000)	Buckwheat, *R. echinopus* (Chmielewski, 2001)
Embryonic development (days)	6.1 (3–10)	4.8 (3–10)	3.4 (3–7)
Hatch larvae (%)	91.0 (50–100)	90.0 (60–100)	97.0 (70–100)
Life history (days)	17.7 (14–25)	19.9 (12–28)	12.9 (10–21)
Eclosion of adults (%)	86.0 (50–100)	80.1 (40–100)	85.0 (50–100)
Frequency of females (%)	70.3 (30–94)	55.7 (33–82)	50.7 (11–60)
Frequency of heteromorphic males (%)	25.5 (0–67)	13.3	
Longevity of imagines (days)	23.8 (10–55)	35.4 (15–104)	33.1 (25–42)
Oviposition period (days)	15.9 (7–39)	16.2 (8–50)	27.5 (22–37)
Preoviposition period (days)	3.2 (2–5)	4.0 (3–6)	2.7 (2–5)
Pause under oviposition period (days)	0.1 (0–1)	0.5 (0–6)	0.3 (0–3)
Postoviposition period (days)	2.6 (0–7)	12.1 (0–72)	3.4 (0–12)
Fecundity per female life-span (eggs)	237.4 (87–628)	221.7 (71–850)	505.6 (233–822)
Productivity of female per life-day (eggs)	11.1 (0–55)	6.1 (0–37)	14.9 (0–53)
Productivity/oviposition-day (eggs)	14.9 (1–55)	13.7 (1–37)	18.4 (1–53)

pus instar); but if the hypopus stage occurs, the developmental cycle may be prolonged even by several months. Each of the instars has a period of activity (feeding, moving) and also for resting, the quiescent period before molting into the next stage, succeeding the development stage. The percentage of specimens that finish their development cycle as imagines, amounts to 86% (on average). In natural populations, females are usually more frequent than males. Newly emerged males and females start to copulate soon after their eclosion. Copulation (retroconiugati position) might be repeated many times during the lifetime of adult mites. This species is oviparous and laying eggs is typical for these mites. Nevertheless, the aparity mentioned above is also sometimes observed. Usually females start their oviposition period several days (3.2 days on average) after copulation. They produce approximately 15 eggs per day and on average, over 237 eggs per their total lifespan, although over six hundred is the maximum. Several days before the natural death of the females they usually stop their oviposition. Adult mites live over 3 weeks (23.8 days) on the average.

Comparison of the present results with biological parameters of *C. berlesei* fed on beebread (Chmielewski, 2000) shows some evident differences, e.g. mites fed buckwheat lived over ten days shorter (average 23.8 days) than on beebread (35.4 days), but their fecundity (avg. 237.4 eggs) is slightly higher than on beebread (avg. 221.7 eggs per female lifespan).

Comparison of the life cycle of *C. berlesei* fed buckwheat sprouts with respective biological data of *R. echinopus* fed the same food under similar conditions show that biological potential of the bulb mite is higher than the potential of *C. berlesei*. That is, the bulb mites live longer (on average 33.1 days) than *C. berlesei* (23.8 days) and their females usually lay over twice of more eggs (avg. 505.6) than *C. berlesei* females (avg. 237.4) under similar conditions (Table 1).

CONCLUSIONS

1) Buckwheat sprouts are a good natural nourisment for *C. berlesei* and can be recommended as an effective medium for rearing and maintaining laboratory monocultures of these mites.

2) This method of rearing of plant fed acarids might be useful in mass production of biological material for acarological experiments and educational purposes.

3) The results show that acaroid mites should be regarded as potential pests of buckwheat sprouts produced as vegetables for consumer purposes.

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Short Communication

Leaf eating weevil, *Strophosomoides kumaoensis* Aslam on buckwheat: a note on damage and biology

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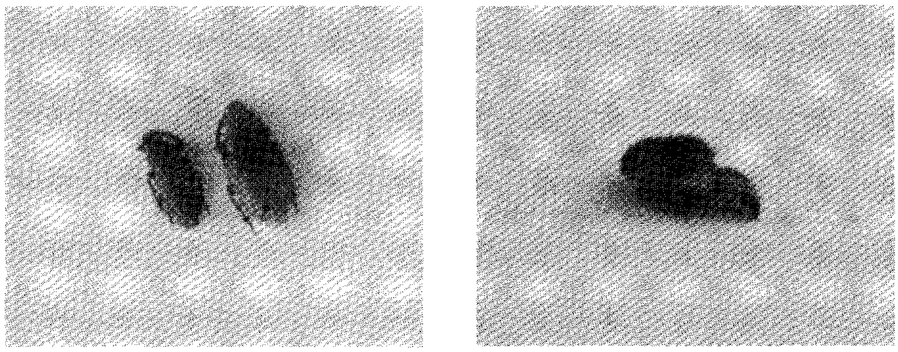
ABSTRACT

Leaf weevil, *Strophosomoides kumaoensis* Aslam has been recorded as a major pest of buckwheat (*Fagopyrum esculentum* and *F. tataricum*) in the Kinnaur district of Himachal Pradesh, India. The pest was earlier recorded on kidney bean or rajmash, *Phaseolus vulgaris* L. from the Sangla valley of Himachal Pradesh. The weevil adults damage the emerging seedlings, young and tender leaves and growing points, whereas the grubs feed on internal root tissues. The reproductive biology and nature of damage of the pest has been clarified.

Buckwheat (Family: Polygonacae), a member of the pseudocereal group, is a source of food grains and have sustained life through the ages in the high hills and mountain regions of India. The two cultivated species of buckwheat, *Fagopyrum esculentum* (common buckwheat) and *F. tataricum* (Tartary buckwheat) are widely cultivated in this region as traditional crop over a long time (Joshi, 1999). The tender leaves as well as the grains are consumed in the form of various dishes and are preferred as food over cereals like wheat, maize and rice. Additionally the grains are fermented to produce local wine.

The buckwheat crop in general is free from the ravage of insect pests (Singh and Thomas, 1978), however during recent years, the crop has suffered heavy losses due to the incidence of a leaf weevil, *S. kumaoensis* Aslam at the experimental farm of Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Research Station, Sangla (Kinnaur), Himachal Pradesh and in the adjoining buckwheat growing areas. The weevil is primarily a pest of kidney bean, *Phaseolus vulgaris* L. (Sharma and Raj, 1998). The pest appears in the second and third week of June on beans and after inflicting losses on the crop, the weevil shifts to the buckwheat crop (sown in June to mid-July). The surveys were conducted one week after germination of the crop in the research farm and adjoining buckwheat growing fields. The extent of damage (percent infested plants) at three random sites in each field was recorded. The weevil infestation on buckwheat ranged from 14–27% and 9–20% during the years 2001 and 2002, respectively. The adult weevils caused damage to emerging seedlings, young and tender leaves and growing points thus hindering plant growth; whereas the grubs (larval stage) fed on internal root tissues and under ground stem portions. Complete defoliation of seedlings in the case of severe infestations was observed and the fields had to be re-sown.

The weevil adults are brownish in colour with strong elytra (Fig. 1a). They are active in the morning and evening hours and seldom appear in the day times (then usually under cloudy weather conditions). The female of the species is larger in size than the male and carries the male on its back while mating (Fig. 1b), which continues for up to 48 hours. To study the biology of the pest, pots filled with sterilized soil were sown with 2–3 seeds and after germination a single seedling was maintained per pot. The pots were kept in the laboratory where the mean temperature ranged between 17–20°C (minimum 11–15°C and maximum 21–24°C), whereas the relative humidity varied from 45–70%. Fifty adult female weevils were collected from mating pairs in the field and one female weevil was released on each plant grown in a pot and caged thereafter. Potted plants were inspected regularly for egg laying and the eggs which were lain on the notched stem portion were counted to work out average eggs laid/weevil. Twenty freshly laid eggs were released in each pot (with a single seedling) and observations on incubation period, larval and pupal period were recorded. A minimum of ten observations were recorded for the larval and pupal periods to work out the mean. Studies revealed that the female laid 4.2 ± 0.67 creamy white, oval shaped eggs singly on the notched stem of the plant, mostly near ground level. Occasionally the eggs were also observed on the upper portions (leaves and stem) of the plants. The eggs hatched within 2.9 ± 0.83 days and the dirty white colored grubs entered the soil. The larval stage lasted for 33.5 ± 1.26 days, with the larvae feeding continuously on internal tissues of the buckwheat plant. The mature grubs are dirty white in color, soft bodied with a brownish mark on the head region. Pupation occurs in the soil in earthen cells near the roots. The



a b

Fig. 1. a: Leaf weevil adults (female and male) on buckwheat leaves and b: Adults in mating (♀ carrying the ♂)

exarate type of pupae is creamish coloured and this stage lasts for 9.3 ± 1.02 days. The total life span varied from 42–56 days in the summer from egg to adult emergence. Adults were observed to be the over-wintering stage of the pest. On review of the literature, no report pertaining to weevil damage in the buckwheat crop could be found. Other species of weevils, *Sagra femorata* (Drury), *S. nigrita* Olivier and *Cypericerus emerginatus* FST. with similar nature of damage on lablab and beans have been reported by Tandon et al. (1975), Nair (1986) and Thakur et al. (1996), but their life cycle differed from *S. kumaoensis*. The pupal period in the case of *S. nigrita* and *S. femorata* is very long, i.e. 4–5 months and 5–6 months, respectively, whereas it is very short 4–6 days in the case of *C. emarginatus*. Moreover no gall formation was observed in case of *S. kumaoensis* as is the case with *S. nigrita*, *S. femorata* and *C. emarginatus*. The weevil population under field conditions could be managed by applying fenvalerate dust @ 20 kg per hectare at the time of sowing or by spraying the crop with synthetic pyrethroids *viz.* deltamethrin 0.0028% or fenvalerate 0.01% or cypermethrin 0.0075% immediately after the germination of the crop.

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