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Buckwheat (*Fagopyrum esculentum* Moench.): Concepts, Prospects and Potential

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ABSTRACT

Buckwheat is grown throughout a large area of Asia and Southeast Asia as a crop. Common buckwheat (*Fagopyrum esculentum*) is a crop of secondary importance in many countries. The crop is not a cereal, but the seeds are usually classified among the cereal grains because of their similar usage. The protein of buckwheat is of excellent quality and is high in the essential amino acid lysine, unlike common cereals. Common buckwheat contains high nutritive substances (63% carbohydrate, 11.7% protein, 2.4% fat, 9.9% fiber, 11% water and 2% minerals). Common buckwheat is also important as a nectariferous and pharmaceutical plant, which contains rutin, its consumption has increased tremendously. The review, which covers the progress, concepts, prospects and potential of buckwheat, is divided into two major parts. The main part covers concepts about genetics, tissue culture, hybridization, self-pollination, molecular markers and proteomics. The section on prospects and potential deals with the development of technologies linked to hybridization and proteomics. Finally, we provide a vision for buckwheat.

Keywords: breeding methods, embryo rescue, molecular markers, proteomics, self-pollinating buckwheat

Abbreviations: 2-DE, two-dimensional electrophoresis; **MALDI-TOF/MS**, matrix assisted laser desorption/ionization-time of flight mass spectrometry; **PCR**, polymerase chain reaction; s^h, self-compatibility gene

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INTRODUCTION

Common buckwheat (*Fagopyrum esculentum* Moench.) is an outcrossing, self/cross-incompatible species belonging to the Polygonaceae family. It is grown mainly in Asia, U.S.A., Canada, Russia and East Europe and its utilization for human consumption is similar to that of cereals. The important component of buckwheat seeds is high quality proteins, containing a well balanced amount of essential amino acids, with a lysine content above 5% (Javornik *et al.* 1981). In addition to its excellent nutritional value, common buckwheat is also important as a nectariferous and pharmaceutical plant. This, coupled with the plant's ability to do well on poorer soils, probably accounts for its widespread usage. It is also a multipurpose crop. The small leaves and shoots are used as leafy vegetables, the flowers and green leaves are used for rutin extraction for use in medicine. The crop produces honey of a very good quality. However, local production is often characterized with persistent low and unstable yields and therefore do not meet demands thus warranting increased important from other nations. The major constraint to buckwheat production world-wide is low seed set and therefore low grain yield. This problem is even compounded by high losses of grains at harvest and

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Invited Review

thrashing as a result of shattering (Fesenko 1986; Wang and Campbell 1988; Alekseeva and Malikov 1992; Ohba *et al.* 1998). Many scientists have identified several production constraints, e.g. Fesenko (1986) and Koblev (1987) identified logging; Ruszkowski (1986), soil quality, weather conditions, pollination frequence, photoperiodism and agrotechnology as factors connected to yielding ability. Kreft (1986) reviewed seed germination, fertility of flowers, and development of kernels, photosynthates distribution and other morphological and physiological problems relevant to the yield of buckwheat. Programs aimed at buckwheat genetic and breeding improvement is further fraught with numerous problems. Prominent among them is the self-incompatibility phenomenon peculiar to the reproductive biology of this seed propagated genus. Other factors hampering effective breeding efforts are apomixis, sterility, low seed set and shattering which are determined by genetic and agro-ecological interactive systems. These breeding barriers have in no small measures contributed to the intractible difficulty and recalcitrance of buckwheat to conventional improvement techniques. For instance, the transfer a valuable genetic trait likes self-fertility and yet another important agronomic character like productivity has so far proved abortive due to cross-incompatibility (Neskovic *et al.* 1995). These phenomena pose a challenge to national research capabilites in increasing buckwheat yields in a sustainable manner. Therefore, novel tools of plant biotechnology such as plant cell and tissue culture that could assist in surmounting breeding barriers in buckwheat was suggested and ever since these techniques have been utilized as an integral approach in buckwheat improvement research. Some attempts to elucidate and resolve some of the enumerated problems of buckwheat genetic improvement in Morden, Canada, KADE Research Ltd. Buckwheat are still produced in significant quantities in many parts of the world. Crop improvement is now being addressed in several countries through collection and evaluation of germplasm.

ORIGIN, PLANT DESCRIPTION, PLANT DISTRIBUTION AND TAXONOMY

Origin

As pointed out by Ohinishi (1995), Nakao (1957) first noticed that De Candolle's (1883) hypothesis of the origin of buckwheat in Northern China or Siberia was contradictory to the distribution of wild *Fagopyrum* species enumerated by Steward (1930). At this point many people believed that the wild ancestor of common and tartary buckwheat was the perennial species *F. cymosum* (Campbell 1976). However, it has been found that the ctDNA (Kishima *et al.* 1995) and the isozyme patterns (Ohnishi 1983) differed greatly between common buckwheat and the perennial species. After the discovery of the species *F. esculentum* subsp. ancestralis and *F. homotropicum* by Ohnishi (1991) it became clear that both species are closely related to common buckwheat and therefore are the probable candidates for the wild ancestor of common buckwheat. As the general trend of evolution in the plant kingdom is in the direction of a self-incompatible outcrossing species to a self-compatible, self-fertilizing species, this trend to eliminate the species *F. homotropicum* as it is a homostylous, self-fertilizing species. This then indicates that the species *F. esculentum* subsp. ancestralis is the wild ancestor of common buckwheat. Therefore, the origin of common buckwheat should be at the location of this species which is the northwest corner of Yunnan province, as this is the distribution of *F. esculentum* subsp. Ancestralis in nature as found to this point (Ohnishi 1995). Li and Yang's (1992) study of the origin of buckwheat supports Ohnishi`s hypothesis that the province of Anion is the area in which common buckwheat originated. Ohnishi (1995) has found several other species in the same area and has developed a map of the distribution of each species.

According to Ohnishi (1995) there are two hot spots where *Fagopyrum* species probably have frequently differentiated. One is the northwest corner of Anion province, the other is the upper Min river valley. In both places there are many different species and all of the new species as found by Ohnishi have come from these areas. The only common wild species that occurs in both areas that has been found to date is *F. cymosum*. This could imply that speciation in *Fagopyrum* might have taken place in these two areas independently (Ohnishi 1995). It is postulated by Ohnishi (1995) that a shift from a self-incompatible outbreeder to a self-compatible self-pollinator occurred independently in several branches of the phylogenetic tree. The finding of the self-pollinating species *F. homotropicum*, closely related to wild common buckwheat, by Ohnishi (1991) at Yonsheng town suggested that evolution from an outcrossing species to a self-pollinating species could have occurred. The subsequent finding of the species at Deqin and Chondian of Anion province and at Luding of Sichuan province (Ohishi and Yasui 1998) showed that the populations were morphologically different. The plants from Yonsheng more closely resemble *F. esculentum* subsp. Ancestralis while the plants from the other locations appear to be more primitive. A shift from an outbreeding species to a selfing one also took place in *F. gracilipes*, where we still can find populations containing both homostylous self-pollinating plants and heterostylous outbreeding plants. In the case of *F. gracilipes*, self-fertilization probably has arisen after duplication of the chromosome number (Ohnishi 1995). It would also appear that the species *F. tataricum*, a homostylous self breeder, originated from an ancestral heterostylous outbreeder. To this point, however, no such species have been found. It is of interest to note that wild and weedy types of *F. tataricum* have been found and as the isozyme patterns very little in tartary buckwheat this would suggest fairly recent speciation. The chances of finding this putative species then appear to be fairly good. The perennial species, *F. cymosum*, has rhizomes and differs from tartary and common buckwheat in its shoots, branching and recemes. From the point of view of isozymes, *F. cymosum* is only distantly related to *F. tataricum* and *F. esculentum* (Ohnishi 1983). Therefore, it is apparent that *F. cymosum* was not the putative ancestor of common and tartary buckwheat as was previously believed. Most of the wild *Fagopyrum* species, including two newly discovered species, have a narrow endemic distribution in southern China. *Fagopyrum gracilipes* and *F. cymosum* are exceptions. The tetraploid *F. gracilipes*, a mainly self-fertilizing weedy species, covers almost the whole of China, except Tibet. Diploid *F. cymosum* has only been found in southern China, however. Tetraploid *F. cymosum* extends its distribution westward to Nepal and Kashmir of India (Ohnishi 1993a). A clear understanding of the evolution of the genus *Fagopyrum* remains unknown. However, it appears that the heterostylous, diploid perennial species *F. cymosum*, *F. statis* and *F. urophyllum* may be prototypes.

Polyploidy has occurred twice, in *F. cymosum* and in *F. gracilipes*. The latter species has almost lost its heterostyly, has become a selfer, acquired colonizing ability and is now widely dispersed. Self-pollination without breakdown of heterostyly appears to be occurring in the Sichuan province of China. The species *F. callianthum* is morphologically heterostylous yet is selfing. Another self-fertilizing new species *F. pleioromosum*, found by Ohnishi, was growing in an adjacent area. True wild tartary buckwheat is distributed in the Sichuan province of China, Tibet, Kashmir and northern Pakistan. By isozyme analysis it can be placed into two groups. One is identical with cultivated tartary and widely distributed. The other, found only in Sichuan, differs at three isozyme loci from cultivated Tartary and is probably an older form (Ohnishi 1991).

The first is a wild form with strong colonizing ability, while the second is more probably a true wild form. A weedy form of Tartary buckwheat with the morphology similar to cultivated tartary buckwheat but having characteristics of wild species including shattering ability and strong dormancy was found in northern Parkistan (Ohnishi 1994). The

Fig. 1 Morphological difference between common (left) and tartary (right) buckwheat.

weedy form may be a hybrid between wild Tartary buckwheat and the cultivated form. The species *F. statice* has existed as herbarium specimens and was described by Wu *et al.* (1984) as distributed in the central part of Yunnan province in China. This species was recently found in Yunmao and Chenjiang of Yunnan province as expected (Ohnishi 1998). Isozyme analyses of *F. esculentum* have revealed that no great allozyme differentiation has occurred among local races in Asia and the centre of genetic diversity in Vavilov's sense is obscure (Ohnishi 1988, 1993b).

Plant description

Members of the Polygonaceae family have leaves that vary in size, arrangement and shape, but the leaf stalk is always surrounded by a membranous or chaffy sheath at the base. The flowers are often grouped in clusters that are showy owing to the colour of the sepals or bracts, for there are no petals. The common buckwheat plant is a broad leaved, erect annual with a single main stem and a brancing habit. In **Fig. 1**, the main stem is grooved, succulent and smooth except at the nodes. The plants generally grow to 0.6-1.3 m tall. The stems are hollow and therefore are subject to breakage by high winds. They are also subject to breakage due to hail as they snap off where struck. They can recover from hail damage by branching from lower leaf axils if the hail occurs when the plants are relatively immature. The plants have a short taproot and fine lateral roots producing a root system that is about 3-4% of the weight of the total plant. The plants can therefore suffer from extreme drought conditions which usually results in delayed maturity. Prior to maturity, the stems and branches vary from green to red. They become reddish brown at maturity. Common buckwheat is an indeterminate species in photoperiodic response. The flowers of *F. esculentum* are perfect but incomplete. They have no petals, but the calyx is composed of five petal-like sepals that are usually white, pink or dark pink. The flowers are showy and densely clustered in racemes at the ends of the branches or on short pedicles that arise from the axils of the leaves. This species is dimorphic, having plants bearing one of two flower types. The pin flowers have long pistils and short stamens while the thrum flowers have short pistils and long stamens. Flowers with pistils and stamens of similar length (Esser 1953; Marshall 1969) and lines with only one floral type (Marshall 1969; Fesenko and Antonov 1973) have been reported. The pistil consists of a one-celled superior ovary and a three-part style with a knob-like stigma and is surrounded by eight stamens. Three of the stamens closely surround the pistil and open outwards, while the other five are closer to the outside and open inward. Nectar-secreting glands are at the base of the ovary. New flower forms such as the one found by Marshall (1969) have short stamens and Quisenberry (1927) reported the inheritance of the flower type in common buckwheat as monogenic. A ratio of 1: 1 of the flower types occurs owing to the incompatibility system. Although genetic control of the flower type, either pin or thrum, appears monogenic, the locus probably is a complex one resembling the model proposed by Ernst (1936). Marshall (1969) found eight distinct classes of style length in F_2 populations derived from crosses between inbred lines of pin flowers with differing style length. He also developed self-fertile lines that bred true for reduced style length. Two sizes of pollen are associated with the heteromorphic system. Large pollen grains approximately 0.16 mm in diameter are produced by thrum flowers while pin flowers produce smaller pollen grains that are approximately 0.10 mm in diameter.

Plant distribution

Although buckwheat is known to have been cultivated in China as early as the 2nd-1st centuries BC (Li and Yang 1992), it is not believed to be very ancient (Hunt 1910). The earliest Chinese records on buckwheat suggest that increased production occurred in China in the 5th and 6th centuries AD (Krotov 1963). Presumably it was cultivated in China for nearly a millennium before it spread to Europe via Russia. It was introduced into Europe in the middle ages, probably from Siberia reaching Germany early in the 15th century (Hughes and Hensen 1934). In Russia apparently it was not grown to any great extent until the 15th century (Krotov 1963). It is not believed that buckwheat was cultivated in Indian ancient times. It was found in India, China and most of Europe in the 17th century. From Europe, buckwheat spread to North America along with immigrants from several countries as it was often used on newly cleared land.

Ohnishi (1988) found that the cultivation of common buckwheat in the Himalayas was limited to approximately 500-2500 m in altitude. Above 2500 m, tartary buckwheat replaces it in cropping patterns. He found a considerable uniformity of allelic frequencies of isozyme loci and suggests that the recent cultivation of buckwheat in this area is a possible interpretation. An alternative and maybe more plausible interpretation is that the history of cultivation in this area is quite long, but that the repeated migration from adjacent populations led to a uniform frequency of allozymes. Migration could have taken place along several trade routes through this area from southern China and Tibet. Ohnishi (1995) found that the places where differentiation occurred were those where a sufficient genetic source for migration had been lacking, that is the margin of the distribution. Buckwheat is believed to have been introduced into Japan about 3000 years ago as archeological evidence suggests (Nagatomo 1984). Buckwheat had already been cultivated extensively as a catch crop when it first appeared in records in Japan in the 8 the century. Nagatomo (1984) and Ohnishi (1995) suggest that buckwheat was introduced to Japan via the Korean peninsula from Northern China. According to Tsukada *et al.* (1986), buckwheat cultivation in Japan dates back to as early as 6600 BP in the Early Jomon period (7000-5000 years BP).

Taxonomy

Buckwheat belongs to the family Polygonaceae. This plant group is generally referred to as the buckwheat, rhubarb or sorrel family. There has been a great deal of interest generated over the past 10 years regarding the classification of *Fagopyrum* species. Much of this has occurred as a result of Ohnishi's work in the finding of six new species in China and his work on their classification. Ye and Guo (1922) suggested a key to the classification 10 species occurring in China. However, the key to the classification of the genus *Fagopyrum* by Ohnishi (1995) is more complete.

CROP IMPROVEMENT BY CONVENTIONAL BREEDING METHODS

Breeding principles

Common buckwheat is a self-incompatible species and this therefore dictates the breeding patterns most used on it. Owing to its outcrossing characteristics, all lines that are being developed must be kept in isolation, either spatial or in cages, from each other. The self-incompatibility of buckwheat is of the dimorphic, sporophytic type and thus seed production is dependent on cross-pollination between "pin" (long pistil, short stamen) and "thrum" (short pistil, long stamen) flowers. Flower forms with reduced style length have been found and self-fertile homomorphic lines have been developed (Marshall 1969). Certain of the lines that were developed were especially adapted to self-pollination since the flowers have equal pistil and stamen heights. However, the introduction of this character into other buckwheat lines almost always results in severe inbreeding depression. This probably due to a large number of deleterious recessive genes being carried along with the thrum gene, as this gene never occurs in the homozygous state. Many breeders have looked to the development of self-pollinating buckwheat as a means of increasing the ease of selection in buckwheat and also as this allows for an extensive search for spontaneous recessive mutations that are normally hidden in the cross-pollinating form. Self-pollinating forms have been reported by Fesenko and Lokhatoba (1981) and Marshall (1969). Fesenko also reports that a study done on Zamyatkin's homostylous long-styled buckwheat form showed it to be a facultative cross-pollinator. It was found that the degree of self-pollination under conditions of free cross-pollination of plants of the same type was 54.5 and 58.6% under field conditions and even higher at 88% when done under greenhouse conditions. This higher seed set under greenhouse conditions may in part be due to the manual pollination that was performed. However, it was shown that the homostylous form was highly self-compatible and capable of self-fertilization not only when individually isolated but when pollen from other plants of the same or heterostylous forms predominated. Induced mutants have been used to increase the polymorphism of buckwheat (Alekseeva 1979). The basic selection method that she used was the individual familial type where families characterized by similar traits and properties are combined and then studied as a single strain. Individual mutants isolated during the breeding process on the basis of definite economically valuable traits and properties or complexes of such traits and properties are studied for varietal testing. She also used biologically valuable mutants in hybridization. She reports that most high-yielding mutants have been produced with the aid of irradiation. The frequency with which desirable mutants appear among forms created by chemical mutagensis is significantly lower. The varieties Aelita and Lada were produced through gamma irradiation in doses of 30 and 40 kR. She also reports that breeding stock obtained through combined treatment of seeds with chemical mutagens and radiation was of particular value. Application of the familial-group selection technique to this stock resulted in creation of the Podolyanka variety. It is interest to note that valuable mutants with high contents of protein and of individual amino acids such as lysine, phenylalanine, methionine, proline, arginine and glutamic acid were found in material subjected to chemical mutagens and to combined chemical and radiation mutagenesis (Alekeeva 1979). Forms that have an increased content of rutin have also been found after mutational treatment. This allows the development of individual desirable traits that then can be introduced into high-yielding or lines with other desirable traits through backcrossing. The improvement of the species *F. tataricum*, although secondary in many breeding programmes, is of major importance in the areas of the world that rely on this crop, these areas being mainly the mountainous regions above 2500 m in altitude that present a danger of frost damage to the crop. Although the species is being evaluated in several breeding programmes for the improvement of common buckwheat, little direct work is taking place on the improvement of this species through crop improvement programmes or by interspecific hybridization. As pointed out by Ohnishi (1995) a putative progenitor species that has a self-incompatible pollination mechanism has still not been found. This would appear to have closely related wild and weedy tartary buckwheat species and types has made it so that crosses are now possible between these species and types. It is of interest to note that in any programme for the improvement of common buckwheat that involves interspecific hybridization with tartary buckwheat, there also exists the possibility of improving tartary buckwheat with very little extra effort. This opens up a broad new area, with its accompanying challenges, in the collaborative breeding of buckwheat. Although tartary buckwheat has several characteristics that are desirable, including frost tolerance and self-pollination, it contains a bitter component that must be removed from any hybrid that utilizes it as a parent. There is a need to determine the bitter component, and to develop a screening technique that can be used in identifying it in segregating progeny.

HISTOLOGY

Pollen tube growth

Inheritance of stylar morphology and loss of self-incompatibility studies in the progenies of induced autotetraploid buckwheat indicated that neither terrible reduction of pollen fertility nor decline in seed set was due to small amount of aneuploids and low frequency of multivalent formation in MI (Adachi *et al.* 1983). Grain yield, therefore increased rather than the original diploid strain and some native varieties. Two types of homostyled variants, namely one reduced style length in pin and the other increased style length were found in the progenies. Of these former plants lost self-incompatibility and therefore set selfed seeds. Pollen tube growth elongation, fertilization and ovule development in autogamous autotetraploid buckwheat revealed that all pollen tubes of illegitimate crosses did not reach the style base. Alternatively, legitimate crosses exhibited high penetration ratio into stylar base (Woo *et al.* 1995). Legitimate combinations in diploids result in 90% normal embryo development both in summer and autumn. In summary, low seed fertility is caused by sporophytic incompatibility due to heterostyly, defectiveness of embryo sac development, and fertilization failure and embryo abortion after fertilization. Based on cross pollen/pistil interaction, pollen tube growth between interspecific hybrids could be classified into 5 categories (Woo *et al.* 1995). There are: 1) Smooth pollen tube growth elongation and stylar base penetration at 6, 12 and 24 hours after pollination. 2) Delayed (for 1 and 6 hours) pollen tube growth elongation. 3) Cassation (for 6 hours) of pollen tube growth. 4) Pollen tube restriction to 50% pistil length. 5) Pollen tube arrest in stigma.

Consequently, fertilization could be estimated by pollen tube penetration indicating that 15 interspecific hybrids could be fertilized.

Developmental analysis of embryo abortion

In a study aimed at resolving the problem of low and unstable yield in buckwheat under unsuitable environmental conditions, it was established that in summer, under high temperature and longer day length, autumn type varieties showed faster vegetative growth and later reproductive development as compared to summer types (Guan and Adachi 1992). In the summer with generally good climatic condition for buckwheat growth in Southern Japan, the embryo sacs and zygotes of both types developed normally. In summer however, mostly abnormal embryo sac were observed 3 days after pollination, leading to incomplete development, failure of fertilization or abortion of embryo. Abnormality of either type occurred in 90-100% of the tetraploids, which is higher than in the diploids (50-77%). This investigation clarifies the factors responsible for low seed set in buckwheat under summer conditions and there provide relevant information necessary for the breeding of adverse environment tolerant buckwheat genotypes. Embryo sac ultra-structural development indicate that high temperatures during anthesis and after pollination within 72 hours result in incomplete development of the embryo sac, abortion of embryo sac and endosperm, degeneration of the egg apparatus, appearance of free stack clusters, accumulation of osmiophilic seposits and the disorder of the organelle (Guan and Adachi 1995). Ultra-structural changes of the embryo at 1, 2 and 3 days after pollination at high temperature indicated that the globular stage of embryo with 6 suspensor cells possessed small vacuoles, endoplasmic reticulum and dictyosomes and presented dense cytoplasm with more abundant mitochondria and plasmids than the suspensor cells. A thin layer of the endosperm with free nucleus surrounded the embryo and part of the endosperm began to form freely growing cell walls. Abortive sterility of the vacuolated embryo sac a major breeding barrier was elucidated. Ultra-structural changes of the embryo sac at 1,2 and 3 days after pollination at high temperature revealed that the globular stage of embryo with 6 suspensor cells contained small vacuoles, endoplasmic reticulum and dictysomes, and presented dense cytoplasm with more abundant mitochondria and plastids than the suspensor cells. Well developed projections appeared in the border wall of the embryo and a thin layer of endosperm with free-nucleus surrounded the embryo and part of the endosperm began to form freely growing cell walls. Abortive sterility in the vacuolated embryo was caused by high temperature.

SOME PHYSIOLOGICAL TRAITS AND THEIR INHERITANCE

Phytotron experiments with four varieties revealed that common buckwheat is sensitive to photoperiodism in the first 20-30 days of growth period in vegetative and floral growth and development respectively. Short photoperiods (9-10 hours) inhibited main stem elongation and favored flowering, whereas long periods (14-18 hours) promoted main stem elongation and suppressed flowering. High temperature (25°C) promoted anthesis whereas low temperature (15°C) restarted the onset of flowering. Segregating progenies from a cross between day-neutral and photosensitive variety showed that flowering in quantitatively inherited with partial dominance of the photosensitive genotype according to Lachmann and Adachi (1990). The electrophoretic property of buckwheat seed protein was investigated by Nishiyama *et al.* (1991) and the SDS-polyacrlamide gel electrophoresis (SDS-PAGE) analysis revealed polymorphism in common buckwheat but not in tartary buckwheat. It was suggested that electrophoretic polymorphism has a relationship to heterozygosity of common buckwheat. To extent of electrophoretic polymorphism deferred depending upon buckwheat cultivar analyzed. A major protein was isolated electrophorectcally from the globulin fraction of tartary buckwheat and its amino acid content determined.

Rutin concentration

There is an increased need to identify and utilize value-added components of buckwheat. This will have the effect of increasing production and thus help increase emphasis on the production of this crop. Rutin was discovered in 1842. Since that time it has been found in at least 34 plant families and 77 plant species. The use of rutin as a medicinal agent for the treatment of vascular disorders characterized by abnormally fragile or permeable capillaries has stimulated interest in this compound. Correction by rutin of increased capillary results in a decreased incidence of vascular complications such as retinal hemorrhage, apoplexy and coronary occlusion (Naghski *et al.* 1955). Rutin has been identified in *Fagopyrum esculentum*, *F. tatricum* and *F. cymosum*. It occurs in concentrations of 3-6% of the dry weight, with *F. tataricum* having the highest concentrations. Although tartary buckwheat was utilized the 1940s for the extraction of rutin it was supplanted by other sources. There now is a trend back to natural sources and a higher concentration of rutin would make the processing of buckwheat more economically feasible. The occurrences and concentrations are not known for the other species of buckwheat that have now been identified. As some of these species are now being crossed with common buckwheat and the remainder may be utilized as sources of specific traits, this information could be of high practical value. Kitabayashi *et al.* (1995) evaluated the varietal differences and heritability of rutin content in the seed and leaf in common buckwheat. Twenty-seven cultivars or strains were evaluated and a wide variation in the mean rutin content was found. The seed rutin content of tetraploid cultivars from Japan at 20.0-22.1 mg/100 g DW was higher than that of diploid ones at 14.5-18.9 mg/100 g DW. However, there was no significant difference between the ploidy levels for leaf rutin content. Also, the varietal differences were significant for rutin content in the seed and the leaf. Highly significant differences in the rutin content of seed were recognized among areas. Some lines from Nepal had higher rutin content in both the seed and leaves, with the seed content being significant. The differences within areas for leaf rutin content were significant for Japanese diploid lines. The heritability of the rutin content in the seed and the leaf was estimated at 0.59 and 0.25, respectively. The results showed that the rutin content in the seed was one of the triats with a relatively high heritability among the main characters in common buckwheat, and that the rutin content in the leaf was a traits comparatively affected by environmental conditions. As

Kitabayashi *et al.* (1995) stated, although a few attempts have been made so far to analyze the varietal differences in rutin content in seeds of common buckwheat, no studies have ever dealt with materials from a wide range of areas in the world. Hurusawa and Harada (1961), who determined the rutin content in seeds of strains collected from various areas of Japan, did not observe any differences among the strains. Ohsawa and Tsutsumi (1993) reported that the varietal differences in rutin content in seeds were significant in 12 strains and varieties mainly from Japan. In the Kitabayashi *et al.* (1995) study it was shown that there was a wide variation in the rutin content among strains evaluated. Some of the Nepalese material was considered to be useful breeding material for high rutin content owing to the considerable variation that was found. The estimated heritability for seed rutin content found was higher than that for 1000-seed weight. Although the rutin content in leaf tissue of common buckwheat has been studied for some time, little is known of varietal differences. McGregor and McKillican (1952) suggested that no significant differences in rutin percentage could be detected in 17 strains tested for a 3-year period. Differences between years, however, were highly significant. It is possible that leaf rutin content could fluctuate because of environmental conditions. A study conducted by Suzuki *et al.* (1987) indicated that the content of rutin fluctuates with light intensity. The grain, however, contains one or more dyes which, as a result of fluorescence are photodynamically active. They can produce an irritating skin disorder, on while or light-coloured areas of skin or hide, under conditions of heavy consumption of buckwheat and exposure to sunlight (De Jong 1972).

PESTS AND DISEASES

Although buckwheat is not attacked by many diseases or pests, a number have been reported on the crop. Flowers are attractive to hoverflies (Syrphidae) (Pears *et al.* 1989): in southern Georgia, Bugg and Dutcher (1989) found adults of the following hoverfly (Syrphidae) species visiting buckwheat flowers: *Allograpta obliqua* (Say), *Pseudodoros clavatus* (Fabricius), *Ocyptamus fuscipennis* (Say), *Ocyptamus costatus* (Say), *Toxomerus boscii* Macquart, and *Toxomerus marginatus* (Say); these are aphid predators as larvae. The following predatory wasps were also observed feeding at the flowers, family and genus or species are given, with known prey items in parentheses: Sphecidae: *Ammophila* sp. (lepidopterous larvae), *Bicyrtes quadrifasciatus* (Say) (Hemiptera), *Cerceris* sp. (Coleoptera), *Isodontia apicalis* (Smith) (Orthoptera: mainly Gryllidae and Tettigoniidae), *Philanthus gibbosus* (Fabricius) (Hymenoptera: Mainly Apoidea), *Prionyx parkeri* Bohart and Menke (Orthoptera: Acrididae, *Pseudoplisus phaleratus* (Say) (Homoptera), *Synnevrus plagiatus* (Cresson) (Unknown), *Tachytes guatemalensis* Cameron (Orthoptera: Acrididae); Eumenidae: *Zethus spinipes* variegatus Saussure, *Rhyacionia frustrana* (Comstock), Nantucket pine tip moth (Lepidoptera: Tortricidae), *Stenodynerus* sp. (lepidopterous or coleopterous larvae), Vespidae: *Polistes exclamans* Vier. (mainly lepidopterous larvae). Also observed were two species of Scoliidae, parasites of Scarabaeidae (Coleoptera), were also observed: *Scolia dubia* dubia Say and *Scolia* (*Scolia*) *nobilitata* nobilitata Fab.

In Massachusetts, Bugg and Ellis (1990) assessed insects associated with buckwheat and four other cover crops: (1) hairy vetch/cereal rye, (2) sorghum, (3) annual white sweet clover, and (4) bell bean. Those workers observed 2 types of Tachinidae at the flowers: *Trichopoda pennipes* (F.) (a parasite of true bugs) and an unidentified species near Archytas (a parasite of caterpillars). Adults of the following aphidophagous syrphids were also seen: *Allograpta obliqua* (Say), *Sphaerophoria* spp., *Syrphus* spp., Toxomerus spp.; and the following non-aphidophagous syrphids *Eristalis tenax* (L.) and *Eristalis* spp.). The following species of predatory wasps also fed at the flowers (family and genus or species are given, with known prey items in parentheses): Sphecidae: *Sphex ichneumoneus* (L.) (Orthoptera: Gryllidae; Gryllacrididae; Tettigoniidae), *Cerceris fumipennis* Say (Coleoptera), *Cerceris* sp. (Coleoptera), *Eremnophila aureonotata* (Cameron) (Lepidoptera: Notodontidae), *Isodontia harrisi* (Fernald) (Orthoptera), *Tachytes pennsylvanicus* Banks (Orthoptera), *Philanthus gibbosus* (Fabricius) (Hymenoptera: Mainly Apoidea), *Philanthus lepidus* Cresson (Hymenoptera: Mainly Apoidea), *Ectemnius lapidarius* (Panzer) (Adult Diptera), *Oxybelus uniglumis* (L.) (Adult Diptera), *Bicyrtes quadrifasciata* (Say) (Nymphal Hemiptera: mainly Pentatomidae and Coreidae); Eumenidae: *Eumenes fraternus* Say (lepidopteran larvae), *Monobia quadridens* (L.) (lepidopteran larvae), *Ancistrocerus antilope* (Panzer) (lepidopteran larvae); Vespidae: *Polistes fuscatus* pallipes Lepeletier (mainly lepidopteran larvae), *Vespula maculata* (L.); Pompilidae: 2 unidentified spp. (Araneae). No Ichneumonoidea were observed imbibing at the flowers of white sweetclover, and few were observed at blooms of buckwheat. This is consistent with observations by Ozols (1964). Insidious flower bug (*Orius insidiosus*), a beneficial predator, and tarnished plant bug (*Lygus lineolaris*), a pest, were relatively abundant on buckwheat on several sampling dates. Also abundant were adult aphidophagous Syrphidae and pooled adult Sphecidae (predatory wasps).

Table 1 List of disease in Buckwheat reviewed by Joshi and Paroda (1991).

Name of Disease	Causal Organism
Brown leaf spot disease	*Ascochyta italica*
Chlorotic leaf spot disease	*Alternaria alternata*
Downy mildew disease	*Peronospora ducumeti*
Leaf spot disease	*Septoria polygonicola*
Powdery mildew disease	*Erysiphe polygoni*
Root and collar rot disease	*Sclerotinia libertiana*
Root and stem rot disease	*Phytophthora fagopyri*
Root rot disease	*Fusarium* spp.
Rust disease	*Puccinia fagopyri*
Smut disease	*Sphacelotheca fagopyri*
Stem rot disease	*Botrytis cinerea*
Stipple spot disease	*Bipolaris sorokiniana*

Table 2 List of pests in Buckwheat reviewed by Joshi and Paroda (1991).

Name of pests	Causal Oranism
Bruchids	*Acanthecelids obtectus*
Cutworms	*Cirphis* spp.
Grain moth	*Cephitinea* sp.
Storage beetles	*Mycetophagus* sp.

Bugg and Ellis (1990) noted that insect visitation to buckwheat was low during afternoons, whereas insects continued to visit white sweet clover. This is consistent with Pellett's (1976) statement that buckwheat often stops producing nectar during the afternoon. Many of these have been reviewed by Joshi and Paroda (1991). The major diseases on buckwheat as reported by Joshi and Paroda (1991) in **Table 1**.

In addition they report that several viruses have been reported to cause reduction in plant height and losses in grain yield. The major pests as reported by Joshi and Paroda (1991) are in **Table 2**.

SELF-INCOMPATIBILITY

Self-incompatibility, due to a condition whereby the plants have short filaments and anthers which are much below the stigmas, has been reported in *F. esculentum*. Self-incompatibility was thought to be restored by single dominant genes (Sharma and Boyes 1961) or possibly by both single and double restorer genes (multiple genes or complex genes) (Woo *et al.* 1997). It is possible that self-incompatibility can be useful in population improvement programs and be used to possibly exploit some of the non-additive gene action on characters affective production. General and specific combining abilities have been estimated in buckwheat for flowers per plant, 100 seed-weight, seed per flower, grain yield per plant. It was generally found that non-additive gene action predominant in both the F_1 and the F_2.

Cytogenetics

Chromosomal and cytogenetic studies have shown the genus *Fagopyrum* to be predominantly diploid with 2n=2x=16 chromosones. The chromosome number of more than 18 species has been reported. Two species *F. esculentum* and *F. cymosum* are tetraploid with 2n=32 chromosomes. These species have been studies cytologically and have been shown to be autopolyploids. Interest in experimental interspecific hybridization in the genus was shown in buckwheat (*F. esculentum*) as early as 1951. Morris (1951) and others were interested in trying to introduce agronomy genes into the cultivated species from wild relatives in the genus. Interspecific hybridization between other species in the genus *Fagopyrum* has been attempted by many researchers since the report of the successful crossing of *F. esculentum* X *F. cymosum* by Nagatomo (1961). Most attempts have been failures and even through many thousand cross combinations are theoretically possible, only seven have been reported as successful in two instances.

In 1951, Morris (1951) succeeded in crossing *F. esculentum* with *F. tataricum*; however this cross has been unsuccessful in subsequent attempts (Samimy 1991). Samimy (1991) reported that *F. esculentum* crosses readily with *F. tataricum* when the latter is used as the female. It was also seen noted that in certain cross combinations fertilization is successful but the embryo aborts during development. The stage of development at which abortion take place differs with the cross combination. Cytological studies of the F_1 hybrids between *F. esculentum* X *F. cymosum* were carried out by Adachi *et al.* (1982), and showed 50-70% chromosome homology and pollen fertility incomformity with the meiotic pairing. From the information available on crossing, fertility, and chromosome behavior of the hybrids, it may be concluded that breeding strategies involving alien genetic transfer for the improvement of common buckwheat is possible through the readily crossable species *F. homotropicum*. There also appears to be a high probability of success in obtaining interspecific crosses between some species that do not cross due to embryo abortion after fertilization through the utilization of embryo rescue techniques. Plant regeneration techniques have been successful in regeneration plants from explants derived from hypocotyl and cotyledon (Woo *et al.* 1997, 2000). The resulting plants showed a high amount of somaclonal variation in plant habit. The technique may be successfully exploited in the production of agronomically desirable types in self-pollinating lines and thus provide a faster means of improvement than allowed by conventional crossing and backcrossing methods. Self-pollinating buckwheat was a very important breakthrough as it clearly demonstrated (Woo *et al.* 1999). Efforts are now under way to modify the self-pollinating plant so that it produces 75% fewer flowers than existing varieties.

Improved lines

In Canada, cv, 'Koban' was developed in 1996, AC Springfield by Springfield Mills Inc., Koma by Kade Research Ltd., Koto in the Morden Research Centre in Canada. It was a bulk of a few plants selected for a locally grown heterogenous population. 'Koban' was a very important breakthrough the high yields and high protein. 'Mancan', a large-seeded diploid variety, has low test weight but good market acceptability; released by Agriculture Canada and licensed in 1974. 'Manor', a large-seeded diploid variety, has low test weight but good market acceptability; released by Agriculture Canada and licensed in 1980. Production of certified seed is limited to Canada. 'Pennquad', a very large-seeded tetraploid variety, has good lodging resistance. The grain is especially well suited for milling because of its large, uniform size, released by the Pennsylvania Agricultural Experiment Station in 1966. 'Tempest', a small-seeded diploid variety with high test weight, and selected by Agriculture Canada from a Russian seed lot and licensed in 1971. 'Tokyo', a small-seeded diploid type with high test weight, and developed by Agriculture Canada from a Japanese introduction. 'Winsor Royal', a large-seeded diploid type with low test weight, but has good market acceptability, and released by Winsor Grain Co., Minneapolis, Minnesota, in 1982. Sale of seed is regulated by the U.S. Variety Protection Act. Cultivars such as 'Botan soba', 'Shinano 1 kou' and 'Hashikamiwase' are the most cultivated and high yielding in Japan. In Korea, cultivars such as 'Yangjeal Meamil', 'Yangjeal Meamil-2', 'Daesan Meamil', 'Dawon Meamil' and 'Deakwan 3-3' are most popular. The principle characteristics of these cultivars are uniform maturity, large black seeds, a protein concentration of 14% and high starch concentration.

CROP IMPROVEMENT BY MEANS OF BIOTECHNOLOGY TECHNIQUES

Embryo rescue

Several attempts have been made to overcome breeding barriers by crossing common buckwheat with some wild relatives. Attempts have been successful in crossing (*in vitro* embryo rescue) between *F. esculentum* and *F. cymosum* tetraploid level (Ujihara *et al.* 1990; Hirose *et al.* 1993; Suvorova 1994; Woo and Adachi 1995; Wang and Campbell 1998). According to Hirose *et al.* (1994), similarity among *Fagopyrum* species, based on pollen tube growth assay, is the greatest between *F. esculentum* and *F. cymosum*, *F. tataricum* is less related to *F. esculentum* and a group of 6 other *Fagopyrum* species is even much more distant. Therefore, it is not a surprise that other interspecific crosses were more difficult to achieve. Such less successful study was for instance published by Samimy (1991) who made attempts for embryo rescue of crosses between *F. esculentum* (pin) and *F. tataricum*. But the reports on successful interspecific crosses with help of embryo rescue technique are absent through some trials to solve this problem were made for example (Wagatsuma and Un-no 1995; Wang and Campbell 1998). Successful hybridization of tartary buckwheat with common buckwheat at the diploid level has been accomplished breeding techniques to cross the two species and then grow the resulting ovules on media. One of the main reasons for the utilization of *F. tataricum* as a parent in interspecific crosses has been the desire to transfer its desirable traits of higher seed yields, self-pollinating ability, frost resistance and overall plant vigor. 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 (1991) has now opened the door to increased interspecific opportunities in buckwheat. Campbell (1995) and Woo and Adachi (1995, 1997) reported on the first successful interspecifc hybridization of buckwheat at the diploid level in which the progeny are fertile and further backcrosses to common buckwheat have been carried out. Using conventional sexual hybridization at the diploid level opens a new area for the improvement of common buckwheat.

Somatic hybridization

Approach towards the genetic improvement of buckwheat by means of biotechnology techniques through plant tissue and cell culture, discovered that hypocotyl segments of common buckwheat and *F. homotropicum* were capable of regeneration by somatic embryogenesis and organogenesis (Lachmann 1991; Woo *et al.* 1997, 1998). Protoplasts isolated from hypocotyls of etiolated seedlings were able to regenerate callus. Mesophyll protoplasts of *F. tataricum*, serving as the hauler (Lachmann *et al.* 1991). The hybrid nature of calli obtained was verified by RFLP analysis. The callus clones analysed predominantly expressed one partial nuclear DNA fragment, as well as the presence of fragments of the other parent in low proportions and novel bands. All the calli analysed carried one parental chloroplast DNA type. The fusion experiments were aimed at the elaboration of simple procedures for overcoming breeding barriers in buckwheat. The physical maps for chloroplast genomes of common buckwheat and its related species (*F. tataricum* and *F. cymosum*) have been constructed using six restriction enzymes (Aii *et al.* 1995; Kishima *et al.* 1995). The genome sizes were identical and estimated at approximately 155.5 kbp. The phylogenic relationship showed that chloroplast DNA of common buckwheat is distant from those of *F. tataricum* and *F. cymosum*. These results that allogamous reproductive system is the possible origin for the genus *Fagopyrum*, because the distantly related species, *F. esculentum* and *F. cymosum*, both maintain self-incompatibility due to heterostyly.

In vitro fertilization

A procedure for isolating cells of the female and male gametophyte in common buckwheat was development (Woo *et al.* 1998a, 1999). Egg cells were separated by enzyme treatment (pectinase 0.75%, pectolase 0.25%, and cellulase 0.5%, pH 5.7, 9% mannitol, 25°C, dark 100 rpm) followed by mechanical isolation. Incubation of ovules for up to 4 hours allowed the boundary wall of the egg cells came out of the micropylar or chalazal end after squashing with a mean isolation frequency of approximately 30%. The isolated egg protoplast is bigger than that of the synergid. Protoplasts of antipodal cells, however, were not obviously smaller than that of egg cells. Sperm cells were released from common buckwheat germinating pollen. After the initial osmotic burst, slurry is sieved through nylon mesh and then subjected to sucrose density gradient centrifugation to remove as starch as possible. The isolated cells are spherical and approximately 5.0-6.0 μm in diameter. The study on characterization of sperm cell using DNA fluorochrome DAPI revealed nuclei nature of the sperm cell. The number of protoplasts and subprotoplasts mainly depend on pollen tube growth. The highest yield of sperm cells was obtained in enzyme solution consisting of 1.0% β-1,3-glucanase (Usukizyme) and 0.25% pectolase enzyme along with mannitol. Cytological observations demonstrated that sperm cells of common buckwheat are true protoplasts, which may allow future fusion experiments to be carried out. The importance of isolated protoplasts of female and male gametophyte is discussed with respect to genetic manipulation and plant biotechnology.

GENETIC RESOURCES

Characterizing material with descriptors

The Germplasm Resources, crop improvement, and Agronomy Committee of the International network for the improvement of *Fagopyrum* have agreed to use following descriptors:

1. First flower-date of first flower in plot.
2. 25% flowering-date when 25% of plants have blossoms.
3. 100% flowering: date when 100% of plants have blossoms.
4. Stand-plant stands per meter of row.
5. Dwarf-rate 0-10 (0 = none, 5 = 50% of plants, 10 = 100% of plants are dwarfs.
6. Flower colour-most prevalent number first (1 = white, 2 = pink, 3 = red)
7. Height-plant height in cm.
8. Maturity-rate 1 to 10 (1 = all green seed, 5 = 50% brown seed, 10 = 100% brown seed).
9. Seed shattering at maturity (0 = no seeds, 5 = 50 seeds, 9 = ≥90 seeds).
10. Plant type (indeterminant or determinant).
11. Plant habit (1 = erect, 5 = prostrate).
12. Seed weight-1000 seed weight in g.
13. Seed density (kg hl^{-1}).
14. Disease-rate 0 to 10 (0 = none, 5 = 50% of leaf area affected by downy mildew, 10 = 100% of leaf area affected).
15. Rutin concentration of the seeds.
16. Dehulled dust, in g.
17. Percent protein.
18. Dehulled weight: in g (including dehulled dust).
19. Test weight: kg m^{-3}.
20. Seed colour-rate 1 to 10 (1 = light brown, 10 = black).
21. Yield: kg of clean seed per plot (noting plot size).
22. Lodging-rate 0 to 5 (0 = no lodging, 3 = 45° angle, 5 = flat)
23. Long cluster: rate 0 to 10 (0 = 0%, 5 = 50%, 10+ = 100% of plants with long clusters)

Dr. C. Campbell, KADE Research Ltd. Morden, Manitoba, Canada coordinates lists of accessions, passport data, and descriptors for all members of the network.

Collections

The collections of buckwheat that take different place throughout the world in the past, have involved large numbers of lines for using breeding purpose. For instance, the Agriculture and Agri-Food Canada, Crop Diversification Centre, Morden, Canada analyzed 3000 lines in 1985 and in 1990. Although these have take place in many different countries and have been accomplished for many different reasons most of them now have one thing in common. The germplasm that was collected has been lost or destroyed and is no longer available to buckwheat improvement programs. While this problem area is now starting to be addressed, an organized system of long term storage with backup storage at other locutions is desperately needed in the areas where buckwheat is now being cultivated. The large amount of variable sample has been found in local germplasm throughout the world, which was described, and stored for future use for improving buckwheat.

Storage and conservation techniques and methods used

The cultivation of common buckwheat is widespread, but in many of the countries were it is produced for home use it occurs in small scale only. As crop improvement programs produce more cultivars the result will probably be more or less genetically uniform cultivars replacing many of the local types. This would be expected in areas where common buckwheat is produced as an economic crop but also have effects in neighbouring regions. This has recently been shown on Hokkaido, Japan where cv. 'Botan Soba' has been replaced with 'Kitawasesoba' over just a few years in some growing regions (Wagatsuma, pers. comm.). Even in areas of small holdings, rapid change can be expected with the introduction of improved types thus genetic erosion can be expected in these areas as well. *Ex situ* collections of common buckwheat are difficult to maintain because of the incompatibility system of the crop. Therefore seed increases must occur under spatial or screen isolation. It is imperative that screen enclosures are double screened to prevent the 'working' of the plants by insects from outside the cages. Large numbers of accessions in some collections put extreme pressures on facilities to accommodate seed increases as plant populations must be large enough to be a true representative sample of the collection and at the same time small enough to be contained in an enclosure (Campbell 1997).

Collections of *Fagopyrum* germplasm are presently being stored in Canada, China, France, Germany, India, Japan, Korea, Nepal, Poland, Russia, Slovenia, Sweden and United States. While many of these are relatively small collections and do not represent a true sampling of the possible genetic variability existing in local germplasm, they are a very valuable component of any buckwheat improvement program.

Inter-specific hybridization in the genus *Fagopyrum*

The wild diploid species *Fagopyrum homotropicum* ($2n = 2x = 16$) has been used for buckwheat improvement, but, prior to this study, the tetraploid form ($2n = 4x = 32$) had not been hybridized with the cultivated species *F. esculentum*. The objective of this study was to hybridize *F. esculentum* with tetraploid *F. homotropicum* to increase the genetic variability. Forty-one interspecific F_1 hybrids were obtained through ovule rescue *in vitro*, with hybridity confirmed using morphological characters, chromosome numbers ($2n = 3x = 24$) and DNA analysis. The F_1 plants were mainly sterile. However, seven seeds were set spontaneously on two hybrid plants, and a large number of seeds were obtained after colchicine treatment. The F_2 plants were divided into two groups based on chromosome numbers and morphology. The first group was hexaploid plants ($2n = 6x = 48$) or hypohexaploid plants ($2n = 44$–46), partially fertile with "*gigas*" features including increased height, dark green leaves, and large seeds with thick seed hulls. The second group of plants was diploid ($2n = 2x = 16$) (one plant had 17 chromosomes), with normal growth and fertility, and a combination of characters from both parents, indicating that genetic recombination had occurred during chromosome elimination. The diploid group was superior to the hexaploid group for use in buckwheat breeding programs due to the desirable characters and the ease of crossing. This is the first report of interspecific hybridization using different ploidy levels in the *Fagopyrum* genus (Wang *et al.* 2005).

Conventional breeding techniques have not been successful in crossing common and tartary buckwheat (Morris 1951; Ruszkowski 1980; Adachi *et al.* 1989). A number of studies on the development of interspecific hybrids have attempted to overcome cross-incompatibility by using embryo and ovule culture systems (Hu and Wang 1986). Successful hybridization of tartary buckwheat with common buckwheat at the diploid level has been accomplished (Wang and Campbell 1998). They used conventional breeding techniques to cross the two species and then grew the resulting ovules on media. Takahiro Wagatsuma from the Horokanai Agricultural Research Center, Horokanai, Japan was the first known person to produce a fertile cross between these two species. He crossed a strain of Chinese tartary buckwheat with a self-pollinating plant obtained from growing var. 'Kitawase' under moisture stress conditions. This cross produced fertile F_1 and F_2 plants which produced viable seeds (Wagatsuma and Un-no 1995). This cross has not been accomplished at Morden, Manitoba with F_2 embryos being obtained to date from F_1 plants with low self-fertility rates. Many interspecific attempts have been made using *F. cymosum* as one of the parents. A successful interspecific hybrid between *F. tataricum* and *F. cymosum*, in the tetraploid state, was reported by Krotov (1975) and named *F. giganteum*. A successful cross between *F. esculentum* and *F. cymosum* was accomplished by Ujihara *et al.* (1990) and Hirose *et al.* (1993), again at the tetraploid level. This was accomplished using ovule culture, further backcrossing of the hybrids with common buckwheat has resulted in fertilized ovules which had to be rescued, with the resulting plants still being sterile. They hybrid was again produced by Suvorova *et al.* (1994) through the use of ovule rescue. Athough the ploidy level was not reported, the hybrids that developed were characterized by complete sterility. One of the main reasons for the use of *F. tataricum* as a parent in interspecific crosses has been the desire to transfer its desirable traits of higher seed set as percentage of flowers produced, self-pollination ability, frost resistance and overall plant vigour. These traits, however, are present not only in this species but also in other species as well. The finding, identification and classification of additional buckwheat species by Ohnishi (1990) has now opened the door to increased interspecific opportunities in buckwheat. Campbell (1995) and Woo and Adachi (1995, 1997, 1998, 1999) Campbell (1995) and reported on the successful interspecific hybridization by conventional pollination of buckwheat with *F. homotropicum* (both at the diploid level, $2n = 16$), in which the progeny are fertile. The progeny have been backcrossed to *F. esculentum* to transfer the desirable traits of *F. homotropicum* to common buckwheat. Isozyme analysis revealed that the tetraploid form was likely allotetraploid rather than autotetraploid in origin due to the fixed heterozygosity and disomic inheritance (Ohnishi and Asano 1999). This hypothesis was confirmed by observations of 16 bivalents at meiosis and 16 homologous chromosomes at mitosis (Wang *et al.* 2002a). As *F. homotropicum* has a self-pollinating system and possesses some frost tolerance, these traits 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 must be eliminated after hybridization. As common buckwheat has a seed abortion problem so severe that often only approximately 12% of the flowers produce mature seeds, the transfer of the self-pollinating mechanism into common buckwheat may allow for increased yield. The interspecific hybrid might also be utilized as a future bridge in further hybridizations with other species. As tartary buckwheat has other desirable characteristics which would be of benefit if transferred to common buckwheat, work has now begun on the development of *F. tataricum* by *F. homotropicum* interspecific hybrids. The successful development of these hybrids has now been made at Morden, Manitoba by KADE Research Ltd. These crosses can now be utilized to transfer traits between all three species. This should not only benefit improvement of common buckwheat but might also benefit tartary buckwheat improvement. This requires that the bitter component be removed and an easier dehulling type developed for those areas of the world that now rely on the hardiness of this species to supply some of their food requirements. Other characteristics of these species might also be transferred, increasing the quality and value-added components of common buckwheat, which could increase the importance and production of a crop that at present is underutilized. The creation of interspecific hybrids between buckwheat species has opened up a new area for plant breeders. These crosses can now provide a means of transferring traits, not available within some species, from one species to another and transferring desirable traits into improved germplasm. The selection of specific desirable traits and the elimination of undesirable ones require a rapid method for the identification of these traits. This would allow for early selection of desirable plants having the specific traits or combinations of traits without having to verify their presence using older and much slower methods. This will thus allow the plant breeder to make much more rapid progress in the improvement of buckwheat and in the development of new cultivars. The identification of molecular markers linked to individual chromosomes and to specific genes has been demonstrated in many species. This usually utilizes random primers in conjuction with polymerase chain reaction (PCR) technology. Primers can be identified that amplify DNA fragments that are polymorphic between lines having different genes as well as being linked to known markers on different chromosomes. This type of analysis, combined with rapid leaf disc DNA extraction techniques, offers a very effective means of applying the knowledge gained to practical plant breeding (Wang *et al.* 2002b, 2005).

INHERITANCE OF HOMOSTYLISM AND HETEROSTYLISM GENES IN BUCKWHEAT

Buckwheat is a cross-pollinated strong self-incompatible, heterostyly plant with two flower forms, namely pin (long styles, recessive homozygote-*ss*) and thrum (short styles, heterozygote-*Ss*), borne on different plants; these features make the genetic improvement of buckwheat very difficult. It is thus not suitable for improvement by conventional plant breeding methods. The majorities of genotypes have an in determinate growth habit (*DD*, *Dd*), and are governed by single genes. Fesenko (1986) studied the gene for the determinant growth in buckwheat and found monofactorial recessive inheritance (*dd*). This inheritance is very good proof of a successful cross. Interspecific incompatibility often occurs unilaterally as a barrier that prevents self-incompatible species from accepting the pollen or the pollen tubes of self-compatible species. Since this unilateral incompatibility has been reported in many families or genera of angiosperms, for example by Lewis and Crowe (1958), Barker (1975) and Ghosh and Shivanna (1984) self-incompatibility has been regarded as an important factor of interspecific cross-incompatibility in many cases. Regarding the genetic model for self-incompatibility, Sharma and Boyes (1961) accordance with Dowrick's (1956) hypothesis of the control of incompatibility by the super gene *S* in Primula. They analyzed the genetic model of loss of function of self-incompatibility in *F. esculentum*. We already reported that interspecific cross-compatibility is closely related to the possibility, cross relationships in the genus *Fagopyrum*, and that suggested 2 groups (compatible and incompatible) and 5 degrees from high compatible to complete incompatible groups had differentiated due to the development of a reproductive isolation mechanism (Woo *et al.* 1995). However, the genetic mechanism of heterostyly gene in buckwheat was always remained of fascinating but unsolved puzzle. Some have even considered it too difficult for scientific research, because the direct evidence is long gone and we can only work by plausible inference. Although this is indeed a very difficult problem to approach experimentally, a number of striking observations over the years have allowed the formulation of plausible scenarios for the genetic nature of various important heterostyly genes. We have tested this strategy in homostyle common buckwheat by screening a single accession of the wild species, *Fagopyrum homotropicum* Ohnishi, a cultivated common buckwheat, *F. esculentum* Moench., for genes capable of increasing the yield and new homostyle appearance (Woo and Adachi 1998). According to Woo *et al.* (1995) and Woo and Adachi (1997), we studied in the F_1, F_2, F_3 and BCF_1 generations. The F_1 hybrids into 1: 1 (homomorphic: thrum type flower plant) indicating a simple dominant gene for homomorphism. Selfed F_2 plant experiments were conduced to define homomorphic gene controlling interspecific crosses and to examine the genetic basis. Homostyly appears to be controlled by a single dominant gene as F_2 progeny segregated into 3: 1 (homomorphic: pin type flower). Homomorphic F_1 plants were selfed, all F_2 experimental lines which were segregated into 3: 1 ratio indicating a simple Mendelian inheritance. Only the F_2 seed with the homomorphic type segregated again in the F_3 generation, with a ratio of 3: 1. But the differences can be observed in nonsegregating progenies and segregating progenies among generations. The chi-square value for joint segregation was not significantly different from the expected value for independent segregation. These results suggest that homostyly genes appear to be controlled by a single locus with multiple alleles. In this study, the author scored the homostylar and the heterostylar loci as if they were segregating 1: 2: 1 instead of 3: 1, this was done because the phenotype of the heteromorphic type was similar to the phenotype of one of the homomorphic type. The BCF_1 lines segregated into 1: 1 ratio expect only 1 pin plant. Thus, BCF_1 (thrum-type, Ss) is easily maintained in the progeny as long as the heteromorphic type is maternally backcrossed with pin-type (ss). On the other hand, backcrossing of the BCF_1 with pollen of *F. homotropicum* gives only self-compatible plants since pin-type, ss, is pollen tube stop stigmas. Accordingly, Ss, ss was used as the recurrent parent in the present experiment to confirm a high transmission of gene derived from *F. homotropicum* in the heteromorphic (Ss/ss) through the pollen. A chi-square test showed the observed ratio did not differ significantly ($P > 0.90$) from the expected ration. From these results, the author estimated tentative genotypes for heterostyly and homostyly in introgressive hybridization. The pin-thrum complex of characters was governed by a single genetic locus S, with three alleles; S and s, which respectively control S^hS^h (*F. homotropicum*), Ss (*F. esculentum*, thrum-type) and ss (*F. esculentum*, pin-type) situation. This situation, corresponding to the incompatibility mechanism in *Fagopyrum*, represents the case of a single locus *S* with three alleles, S, S^h and s, and two phenotype, pin and thrum. It may be represented by relationship of dominance, $S > S^h > s$. As the results, the proposed style genes model is that the homomorphic, S^hS^h, allele induces pollen tube stop in stigma carrying the ss allele in the heteromorphic (thrum-type Ss, pin-type ss), whereas the S^hS^h allele induces no pollen tube stop in stigmas with either of the other alleles. All of the homorphic (S^hS^h) are self-compatible so that self-incompatibility occurs only in the hybrid between S^hS^h, ss and ss, S^hS^h. However, the S^hS^h, ss F_1s are generally self-incompatible. The results observed in the crosses of Ss, ss, ss, Ss and S^hS^h, S^hS^h showed that a single gene is responsible for self-compatibility. But, not that S^hS^h induces the pollen tube stop in stigmas with Ss in the heteromorphic (Ss, ss). A similar genetic explanation was reported on the development of a self-compatible in interspecific hybrids derived from *F. esculentum* (pin) and *F. homotropicum* (Campbell 1995). The F_2 progeny segregated into 3: 1 ratio for both and flower types of self-compatibility cosegregated completely with the homomorphic phenotype. This indicated that the homomorphic flower type and self-compatibility were either governed by a single dominant gene or two closely linked genes. This study indicated that homostyly also was controlled by a single dominant gene similar to several other genetic analyses of morphological, physiological and developmental traits and isozyme variability in common buckwheat (Ohnishi 1990). The occurrence of homomorphic shapes and pin-flower types in interspecific hybrids between *F. esculentum* (thrum-type) and *F. homotropicum* are reported here for the first time. The new gene has no pleiotropic effects and all other characters of the plant essentially remained unaltered. Symbol S^h is proposed for the homostylar. The homostylar and the heterostylar seem to be important to plant breeders as genetic marker in isolating accidental selfing during hybridization, and in identifying and evaluating marker linked genes affecting specific quantitative traits. As a marker it also may be of value in surveying genetic variation in populations and interpopulation variability. The experimental results show interesting questions as follows;

1) Why did a significant difference in pollen-pistil interaction happen between different styles, pin and thrum in *F. esculentum* crossed with homostylous species *F. homotropicum*.

2) How the author can exploit the wild annual species, *F. homotropicum* to enhance genetic diversity in buckwheat.

METHODS USED FOR BUCKWHEAT BREEDING

Tartary buckwheat (*Fagopyrum tataricum*) is a highly nutritious crop that is widely grown in Asia, but the flour contains a large portion of the hull if it is ground with industrial processing since the hull is very hard to remove as it adheres to the testa layer of the groat. Rice-tartary, a particular type of tartary buckwheat, has seeds with a loose hull and the presence of splits on the sides of the seeds that make dehulling easily. The first attempt of crop improvement in tartary buckwheat is breeding program through hybridization. Hybrids were obtained by hybridization of crosses between tartary buckwheat and rice-tartary. Additional crosses were made among selected progenies of these crosses. Based on progeny analysis of the F_1, F_2, and F_3, the character of rice-tartary, as exhibited as the presence of splits on the sides of the seeds, is controlled by one gene which is homozygous recessive. A tartary buckwheat breeding program has been conducted for 6 years based on these crosses. Advanced lines with easy dehull and yield potential are at the stage of ready for yield trials. Production of easy dehulling tartary buckwheat in the future could boost Canada's domestic and international markets (Wang e*t al.* 2007).

Buckwheat breeding is a recent endeavor when compared with breeding efforts devoted to cereals or some of the other health foods. Common buckwheat still occupies most of the cultivated land sown to buckwheat in the major producing countries. Moreover, most of the cultivars released to date have been derived from selection within heterostyle common buckwheat and are not the results of hybridization. Nevertheless, it is expected that new and improved cultivars will become available in many buckwheat-producing areas due to the increased efforts in buckwheat breeding in both national and international programs (Campbell, unpublished).

The methods of breeding buckwheat are pure line selection or hybridization followed by the bulk method, the pedigree method, the single seed descent, or some modification of these procedures.

Pure line selection

Mass selection and pure line selection within introduced germplasm accessions or common buckwheat have been the principle methods of buckwheat improvement in developing countries. Also, as the buckwheat crop has been introduced to relatively new production areas (United States and Canada), the initial cultivars used were developed from selection within introduced common buckwheat. Genetic variability in the germplasm collections worldwide has not been fully exploited to improve important agronomic traits.

Bulk population

Bulk population breeding has been the preferred method for buckwheat because of its ease of application and because of the problems involved with alternative methods. The method is simple, requires minimal record keeping, and is not labor intensive. Simplicity makers it attractive for programs that are designed to develop cultivars adapted over a wide geographical area because subsamples of populations can be widely tested. The advantage of wide testing includes the possibility of natural selection favoring genotypes adapted to particular local environments. However, caution is needed when using the system to ensure the survival of desirable genotypes through successive generations of bulking. It is expected that seed size differences between parents and the larger numbers of seeds produced on small-seeded genotypes compared with those on large-seeded genotypes may cause rapid shifts in a bulk population toward a preponderance of small-seeded types. To increase the proportion of desired phenotypes, populations can be subjected to mass selection either on the basis of seed size or color or on plant traits such as, for example, flowering time, plant height, branching characters, or disease resistance. Selection in early generations, such as in the F_2 to F_4, might be effective in eliminating many undesirable genotypes. Selected plants can then be grown in bulk for several generations, followed by reselection after the populations have reached homozygosity. Because of the problems of genetic shifts during generation advance, modifications of the bulk population method have been devised. These include mass pedigree, modified bulk, single seed descent, and other schemes designed to control genetic shifts or to channel the shifts in the desired direction. Kade Research uses a bulk pedigree method in which crosses are advanced in bulk to the F_4, after which the pedigree method is used. The generations advance by bulking, which allow an early evaluation and selection of bulks on which to concentrate efforts. Selection of plants in the F_4 is based only on highly heritable plant characters, and thereafter the progenies are managed by the pedigree method. Visual selection of F_4 plants according to available selection criteria should lead to greatly improved types.

Pedigree selection

The pedigree method of breeding is not the choice of most buckwheat breeders for managing buckwheat breeding material. However, if the method is used, between 5 and 15 F_1 plants are sufficient to provide from 600 to 6000 F_2 seeds. To allow for successful selection, buckwheat plants needs to be widely spaced so that individuals can be observed for possible selection. The plastic branching habit of buckwheat plants is a disadvantage of this method because when widely spaced for observations, their performance may be entirely different from that in more densely sown stands as used in commercial production. Also, for pedigree selection to succeed, readily identifiable traits need to be available, which is not the case in buckwheat. However, selection of the F_2 plants for traits with large heritability estimates (such as flowering date, relative maturity, and seed size) is likely to be successful. Characteristics that are considered desirable in buckwheat, such as elect growth habit, greater branch number, earlier flowering, and suitable maturity dates, should be readily distinguishable among F_4 families. The F_7 provides the first opportunity to observe selections in comparison to standard check cultivars. Selected F_7 lines are usually sown in multi-row plots that have within- and between-row spacings similar to those used in farmer's fields. Preliminary yield trials may also be conducted. With this approach, line characteristics can be observed in solid sowings and yield potential can be gauged. Lines that meet the selection criteria of plant type, relative earliness, degree of branching, seed size and color, and yield are then retained and entered into advanced yield trials in succeeding generations.

In Canada, KADE Research Ltd. has proposed a modification of the bulk method in which individual F_2 plants are selected and evaluated for yield in the F_3 and later generations. The method, designed as the "F_2-derived family method" places early emphasis on yield potential with the expectation that genes for yield can be actively selected for in early generations (Campbell, unpublished).

Backcross method

The genes for resistance to wild species and cultivated species similar simply inherited genes in buckwheat are well suited to introgress to acceptable cultivars by means the backcross method. Good sources of resistance are available and resistant plants are easily identified in segregating populations. Nevertheless, the backcross method has not been widely used in buckwheat improvement programs.

MOLECULAR MARKERS

An important aspect of genetic research is creating genetic maps that are useful to geneticists and plant breeders. DNA markers can be employed in the construction of genetic maps, which help determine the chromosomal locations of genes affecting either simple or complex traits (Paterson *et al.* 1991) with these molecular methods, genetic maps of diploid plants can be developed mare rapidly than those of ployploids. The origin of cultivated common buckwheat has been studied by the analysis of the diffusion routes of cultivated common buckwheat using RAPD markers (Murai and Ohnishi 1996), by the determination of the allozyme diversity of the cultivated populations (Ohnishi 1998) and by the analysis of the genetic relationships between cultivated populations and natural populations of wild common buckwheat using AFLP markers (Konishi *et al.* 2005). During the course of these studies, several issues that were difficult to solve by the use of allozymes as well as of RAPD and AFLP markers, were identified, for example, the determination of the center of genetic diversity, detailed genetic relationships among cultivated populations, and gene flow between cultivated populations and natural populations of wild common buckwheat. If more powerful markers became available, it would be possible to address these issues, so that studies on the origin of cultivated common buckwheat could progress more rapidly. Using RAPD, Aii *et al.* (1998) constructed a linkage map of buckwheat. The RAPD map so generated is relatively dense, with a 0.6 cM distance between markers. The mapping of this gene represents a first step toward better understanding of sexual reproduction system in buckwheat. These markers should be useful for marker-assisted selection for introgression of self-compatibility into common buckwheat. Also, using molecular markers, Aii *et al.* (1998) have cloned and sequenced three RAPD fragments tightly linked to the Sh gene in buckwheat. Nucleotide sequence information, obtained from each end of the three linked RAPD markers, was used to design 21-mer oligonucleotide primers for PCR amplification of the respective SCARs. Two of these SCAR primers, SCB14 1250 and SCP8 1000, amplified a single fragment in *Fagopyrum homotropicum* but was absent in common buckwheat, *F. esculentum*. In one case, SCQ7 800, showed different PCR fragments between common buckwheat and *F. homotropicum*. This co-dominant marker would be useful for differentiating between heterozygous and both types of homozygous forms. Recent advances in molecular biology have offered a suite of molecular tools for assessing genetic diversity. Among the PCR-based techniques, amplified fragment length polymorphism (AFLP) (Vos *et al.* 1995) and simple sequence repeat (SSR) markers are widely used for studies of genetic diversity in crop species. Although AFLP markers have two weaknesses, a dominant mode of inheritance and the fact that homology is inferred from band comigration, they have large advantages, namely the ability to resolve a large number of loci from a single reaction and the fact that no prior sequence information is required. The advantages of SSR markers are their codominant mode of inheritance and hypervariability, which make them ideal for a wide range of applications (Goldstein and Schotterer 1999). However, the development of an SSR assay requires the laborious processes of library construction, DNA sequencing, and primer synthesis. No SSR markers for common buckwheat were available previously. Iwata *et al.* (2001) who developed SSR markers for common buckwheat, observed a significant genetic differentiation among Japanese common buckwheat cultivars (Iwata *et al.* 2005). However, only five SSR markers were developed by Iwata *et al.* (2001). Nagano *et al.* (1998) used AFLP and bulked segregate analysis to develop molecular markers linked to the self-compatibility (S^h) gene. AFLP provides many genomic bands for buckwheat. 62 bands specific to homomorphic flowered plants were detected. AFLP should be useful for marker assisted selection for introgression of self-compatibility into common buckwheat and also for detailed analysis of plant genomes. To further enrich genetic resources of buckwheat, Yasui *et al.* (2008) constructed a bacterial artificial chromosome (BAC) library. In many species a large insert genomic library, such as BAC, P1 artificial chromosome (PAC) or transformation competent artificial chromosome (TAC), has been indispensable, not only for genome analyses and functional studies of genes of interest but also for studying the evolution of genome or genetic systems of organisms. Notably, buckwheat belongs to Polygonaceae, one of the core tricolpates that diverged from other major tricolpates at an early stage in angiosperm evolution, and buckwheat genomic library would be served as a useful resource for the study of plant genome evolution.

APPLICATION OF PROTEOME ANALYSIS FOR BREEDING

A panel of powerful genomic technologies now permits the determination of complete genome sequences and the global measurement of mRNA gene expression profiles in cells. It is the promise of proteomics that 1) the data obtained from genetic and genomic techniques by themselves are insufficient to describe physiological and pathological processes and that 2) the data obtained from the global analysis of gene expression at the protein level (proteome analysis) provide a better or complimentary basis for the analysis of biological processes. Proteome analysis is performed to make the best use of the data obtained in the genome research project according to the following steps. (1) A number of proteins are separated by mainly two-dimensional electrophoresis (2-DE). (2) Based on the peptide-mass fingerprint and partial amino acid sequence, the proteins separated by 2-DE are identified by the use of protein database, and simultaneously the genes encoding them are identified in the gene library constructed in the genome project. (3) The proteins without known function are characterized in amount, localization, structure, post-translational modification, localization, enzyme activity, protein-protein interaction, etc. (4) The proteome database is constructed. (5) The functions of a number of proteins and genes are determined based on the data obtained from both protein and gene analyses. This step seems to be most important and difficult in proteome analysis.

Cleveland mapping peptide techniques improved for efficient proteome analyses (Woo *et al.* 2002). Also, a total of 105 proteins separated by 2-DE were analyzed by peptide mass fingerprinting using MALDI-TOF/MS (Fukuda *et al.* 2003). Although peptide mass fingerprinting is a sensitive high-throughput method, the rice proteins or genes could not always easily identify by the mass fingerprinting. This article is focused in developing the techniques for deblocking of the *N*-terminally blocked proteins separated by two-dimensional gel electrophoresis. Using the developed Cleveland mapping peptide techniques, 40 buckwheat embryo proteins and 100 seed proteins on 2-DE and electro blotted them on to PVDF, analyzing and determined the partial amino acid sequences, were analyzed by Edman degradation using a gas-phase sequencer. The *N*-terminal sequences of 1 protein were determined (Woo *et al.* 2001, 2006). In addition, the proteome analysis will pursue the database approach to study a specific stress physiological process, pathological process and plant diseases state of an organ and tissue. There are still many additional challenges that will have to be addressed before complete proteomic projects can be implemented to the study of plant stress and diseases. The obtained knowledge allows us to regulate the developmental process and stress process for the buckwheat and finally to breed new varieties with high yields in near future.

Recently, the peptide-mass fingerprinting by matrix assisted laser desorption ionization time-of-flight (MALDI-TOF) MS has often been used for the identification of proteins and genes. Although MALDI-TOF/MS is a high throughput method, it is not always useful to identify the proteins and genes in plants as well as in the other eukaryotes. Since plant proteins are often post-translationally modified, this makes it difficult to identify the proteins and genes by the peptide mass fingerprint. Mass Spectrometry (MS) such as electro-spray ionization quadrupole-time-of-flight MS and be used to determine the partial amino acid sequences of proteins separated by 2-DE (**Fig. 3**). This is a highly sensitive and accurate, but not high-throughput method. Gas-phase sequencing is neither rapid nor sensitive compared with MS in identification of proteins, but still useful to identify proteins separated by 2-DE. Because, in this system, a relatively small (pmole) amount of proteins can be precisely identified based on the sequences obtained (Park *et al.* 2009; Woo *et al.* 2009).

According to Park *et al.* (2009), the proteins from single seeds revealed distinct variation among the total seeds investigated. In **Fig. 2**, the differences in banding pattern can be characterized into three areas on the electrophoregram (1a-4a - 54-47 kDa); (1b-7b - 45-24 kDa); (1c-3c - 16-11 kDa). A variation of 29.68% was found among the protein subunits between 47 to 54 kDa. While the 45 kDa showed variation among 32.5% of the total seeds tested, 17.8%

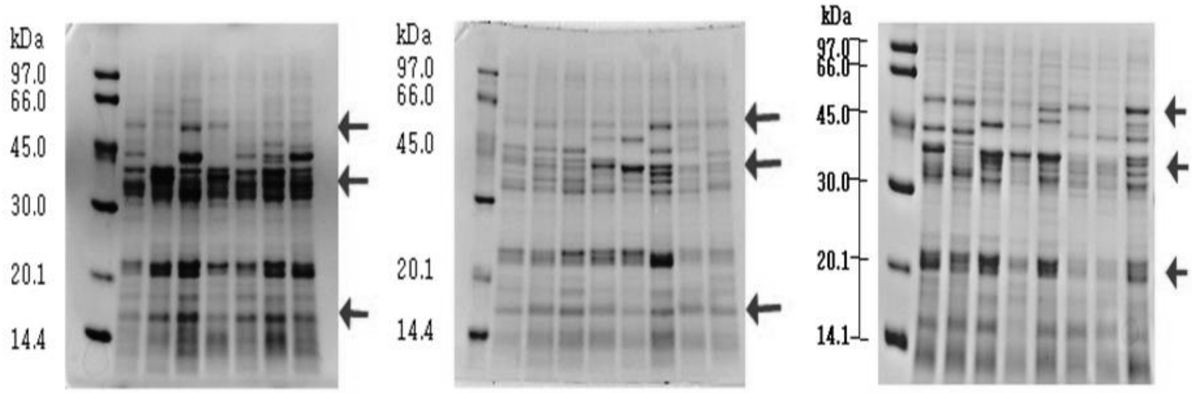

Fig. 2 SDS-PAGE of buckwheat water soluble common buckwheat seed storage protein. Arrowheads show polymorphism.

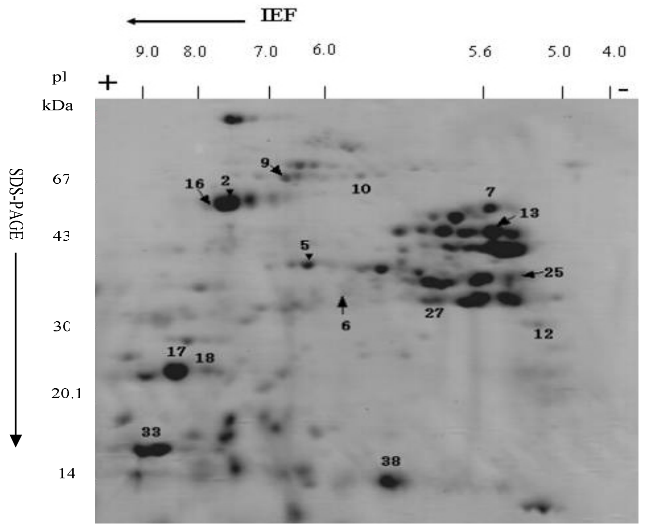

Fig. 3 Two-dimensional gel patterns of the embryo proteins of common buckwheat. First dimension (left to right), isoelectric focusing; second dimension (up to down), SDS-PAGE. The numbered spots were excised and analyzed by Edman degradation. Major polypeptide spots (2, 17, 38) to already known proteins according to their internal or *N*-terminal amino acid sequences, are numbered.

showed variation for 40-42 kDa protein subunits. The protein subunits of 38 and 32 kDa showed variation to the extent of 23.43%. The 28 kDa protein subunit showed variation of 11.25%, while the protein subunit 24 kDa was not present only in 8 seeds out of 160 seeds tested. The 16 and 13 kDa protein subunits showed 6.25% variation and the 11 kDa protein subunit showed 13.12% variation (**Figs. 2, 3**). We identified 24 kDa subunit from single seed, interesting results were obtained on the spectrum of variation among the seeds of common buckwheat cultivar Suwon No. 1. The 24 kDa subunit was reported as allergic protein subunit by Urisu *et al.* (1995). The intravarietal heterogeneity for different subunits was also reported by Svetek (1994). The major class of buckwheat protein was reported as 13s globulin by Svetlana *et al.* (1996). The 13s globulin on SDS-PAGE revealed protein subunits from 43 to 68 kDa and another fraction between 14 to 23 kDa. The maximum polymorphism was revealed among these globulin subunits. Variation among 11 to 16 kDa indicates that polymorphism also exists amongst other protein fractions of buckwheat.

PRESENT CROP IMPROVEMENT PROGRAM

Many crop improvement programs are presently addressing research on the different aspects of buckwheat production. Several of these are programs that contain a number of different, but interrelated, fields of specialization. These developments along with increased “marketing” and collaboration with other programs have provided a good base for rapid improvement in this formerly badly neglected crop. The various facets that are contained in different programs include not only crop improvement aspects but also those of a nutritional, medical, socio-economic and biotechnical nature. Increased interest has been shown for human consumption of tartary buckwheat due to some of its components that are very beneficial to human health. This has created increased crop improvement programs in China, Japan and Canada. This increased breeding effort on this species would be expected to increase in the future and result in a second buckwheat species that will become increasingly commercially important on an international scale (Campbell 2004). Some of the major crop improvement programs and their major emphasis on present research are listed below. The *Fagopyrum* improvement program at the Agriculture and Agri-Food Canada, Crop Diversification Centre, at collaboration KADE Research Ltd. Morden, Manitoba, Canada, the program includes aspects of self-pollinating for increased yield, frost resistance for longer growing season at less risk, increased seed density for greater flour recovery, and nutraceutical components for increased value. Grand Forks Human Nutrition Research Center conducted “Nutritional value of buckwheat: trace element variability and bioavailibility and fagopyritol content” project (2003-2006). Agriculture Research Service under United State Department of Agriculture take different initiatives for Buckwheat research such as breeding program, germplasm collection and nutritional quality improvement.

FUTURE BREEDING STRATEGY AND PROSPECTS

Many of the crop improvement programs will continue with the main emphasis being on the development of high yielding, adapted cultivars containing very high amounts of the rutin and zero allergic protein. Attempts will be made to high amounts the rutin and completely remove the allergic protein content addressed by two main approaches. Identification and purification of the enzyme responsible for allergic proteins production, and by transfer of genetic characters through inter-specific hybridization utilizing both cytogenetics and tissue culture techniques. The feasibility of introgression of desirable characteristics from other closely related species will be also be addressed. The emphasis on seed yield will probably remain, and forage aspects on this crop are starting to demand more attention. Enhancing the nutritional value of the crop by reducing anti-nutritional factors will also receive more emphasis. Many of the crop improvement programs will add another dimension or directions to their present programs. In future research at Morden, Canada, for example, investigations will be conducted along four directors; Breeding of reduced allergic protein lines with good agronomic characteristics will continue as well as crop improvement by self-pollinating and frost resistance for longer growing season at less risk. These aspects are now increasingly being addressed by several programs.

1) Select genotypes with increased herbage production, seed yield, and harvest index.
2) Assess the selected genotypes for tolerance to production constraints (leaf diseases, drought, and cold).
3) Develop other important traits (earliness, leaf-retention, erect plant habit and non-shattering seeds).
4) Self-pollinating for increased yield but low allergic protein content.
5) Target suitable *Fagopyrum* species to specific farming systems.
6) Identify potential frost-resistance.
7) Increased green test lines
8) Increased rutin content
9) Increased seed density for greater flour recovery (change the starch functionality).
10) Nutraceutical components for increased value.

Molecular genetics has emerged as an applied research discipline during the past decade and promises to assist in the solution of many agricultural problems. Genetic engineering holds promise for development of specific genotypes since it is based on identification characterization, and transfer of specific genes into the recipient plant as compared to the mixing of two complete genomes of two parental lines followed by backcrossing for several generations to remove undesirable genes. Molecular genetic technologies can be employed to characterize the genes and identify important linkage which could facilitate gene transfer to suitable agronomic types, genes for disease, insect resistance, frost resistance, reduced allergic proteins concentrations, increased protein content, and other characters of agronomic importance seen to be adaptable to this approach. Interdisciplinary research efforts, now underway at several research establishments together with collaboration between institutes, promises to produce needed improvements in buckwheat in the near future.

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