Interspecific Hybridization and Genetic Studies in Buckwheat (*Fagopyrum* spp.)

BY
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A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY

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INTERSPECIFIC HYBRIDIZATION AND GENETIC STUDIES IN BUCKWHEAT (*FAGOPYRUM* SPP.)

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A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of

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ABSTRACT

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The interest in the wild buckwheat species *Fagopyrum homotropicum* is primarily as a potential source of self-compatibility trait, which could be used to improve the yield stability of the cultivated species *F. esculentum*. Interspecific hybrids between *F. homotropicum* and the two cultivated species, common buckwheat (*F. esculentum*) and Tartary (*F. tataricum*), were produced using ovule rescue technique. This success is not only useful for buckwheat improvement but also creates the possibility of use *F. homotropicum* as a bridge species to achieve the successful hybridization between the *F. esculentum* and *F. tataricum*, as directly crossing the two species is difficult. The allotetraploid origin of *F. homotropicum* (2n = 4x = 32) form is supported based on fertility and karyotype by comparing the tetraploid with the diploid form of *F. homotropicum* and with *F. esculentum* spp *ancestrale.*

The fertility of interspecific hybrids between *F. esculentum* and *F. homotropicum* 4x was restored by colchicine treatment or by the occurrence of spontaneous doubling. The progeny had either approximately double the F_1 triploid (2n = 3x = 24) chromosome number (2n = 6x = 48) or the reduced chromosome numbers (2n = 2x =16). These progeny with recombinant morphological characters from both parents provide valuable genetic resources for buckwheat improvement and genetic study.

Several alternative approaches were used to produce interspecific hybrids of the two cultivated species, common buckwheat and Tartary. These strategies included the direct cross following by chromosome doubling, the multiple cross combining the two single crosses, the bridge cross using *F. homotropicum* to connect the two cultivated species and further backcrossing of the bridge hybrid with the self-pollinated buckwheat. Even though the hybrids were sterile, pollen viability was significantly improved from 1.7% in the direct cross to 21.8% after backcrossing bridge hybrids with the self-pollinated

buckwheat. This result is promising because fertility may finally be restored by repeated backcrossing.

Three genetic studies in this thesis were conducted using interspecific hybrids between *F. esculentum* and *F. homotropicum* 2x as well as intraspecific hybrids within *F. homotropicum* 2x.

Self-compatibility is shown to be controlled by two complementary dominant genes with a three allelic series, S, S^h, and s at the first locus that controls the anther height relative to the style to produce thrum homostyly, and pin phenotypes. The S allele is dominant to S^h and s, and S^h is dominant to s. The second locus has two alleles, S_c and s_c with the S_c allele for self-compatibility (homostyly) dominance to s_c for self-incompatibility (pin). The genotype of self-compatible *F. homotropicum* was proposed as $S^hS^hS_cS_c$, while self-incompatible *F. esculentum* has more than one genotype with the first locus fixed, ssS_cS_c, ssS_cs_c or sss_cs_c producing the pin flower type and SsS_cS_c, SsS_cs_c or, Sss_cs_c producing the thrum phenotype.

Seed shattering is dominant to non-shattering and controlled by three complementary genes. The heterozygous genotypes of *F. esculentum* resulted in three segregation patterns, 3:1, 9:7, and 27:37, in the F_2 populations.

The trait of winged seed is dominant to non-winged seed and is controlled by two genes. As one of the loci in *F. homotropicum* is fixed as homozygous dominant, inheritance of winged seed within *F. homotropicum* behaved as if it was controlled by a single gene. However, when *F. homotropicum* plants with winged seed were crossed with the plants of *F. esculentum* with non-winged seed, the F_2 progeny produced either 3:1 or 9:7 ratios as the second locus in *F. esculentum* were not fixed as homozygous dominant.

FORWARD

The manuscripts were formatted according to the journal of Crop Science.

1.0 INTRODUCTION

This study focused on the application of interspecific hybridization among three species of buckwheat to explore the potential of improving the two cultivated buckwheat species *Fagopyrum esculentum* and *F. tataricum*, including the integration of traits from the wild species *F. homotropicum*.

The major production challenge of *F. esculentum* is yield instability, mainly due to self-incompatibility, susceptibility to frost, and sensitive to environment stresses. In contrast to *F. esculentum*, *F. tataricum* is a self-compatible species with frost tolerance. However, the flour of *F. tataricum* has a bitter taste and seed is small with a tight seed coat that makes dehulling impossible. It is possible to improve both species through the recombination of characters in interspecific hybridization. Since Morris (1951) first attempted the interspecific cross between the two cultivated species, only one sterile hybrid from 263 ovules rescued *in vitro* was reported by Samimy et al. (1996) without fertility restoration. Numerous researchers have repeated the same cross (Fesenko, I. N. et al., 2001; Chen, 1998; Hirose et al., 1995; and Woo, 1998) without success. However, there has been no effort to cross the two species using different strategies such as multiple crosses or bridge crosses as in order to produce successful hybrids. These techniques should be attempted to establish the feasibility of the breeding approaches.

Fagopyrum homotropicum is a wild species with self-compatibility, frost tolerance, and other desirable characters that could be used in the improvement of *F. esculentum*. However, the difference between diploid and tetraploid forms of *F. homotropicum* has not been studied with the exception of isozyme analysis (Asano, 1998). The use of *F. homotropicum* to improve *F. esculentum* has been limited to interspecific hybridization between *F. esculentum* and the diploid form of *F. homotropicum* (Campbell, 1995). No studies have been published on the crossability, hybrid production, and cytological characterization in intraspecific hybrids within *F. homotropicum* and interspecific hybrids between *F. homotropicum* at both the diploid and tetraploid level and the two cultivated species. It is assumed that *F. tataricum* crosses with *F. homotropicum* more easily than with *F. esculentum* because three species in the same phylogenetic group, the cymosum

group, and *F. tataricum* and *F. homotropicum* have the same breeding system, distyly and self-compatibility. If this is true, *F. homotropicum* is not only useful for common buckwheat improvement, but can also act as a bridge species for hybridization of the two cultivated species. At the same time, crossability data will be valuable in determining the phylogenetic relationships among *F. tataricum*, *F. homotropicum*, and *F. esculentum*.

It is important for breeders to understand the inheritance of the two breeding systems in buckwheat species, self-incompatibility with distylic flowers and self-compatibility with homostyly, particularly when hybrids are developed from the two species with the two systems. Previous studies of self-compatibility inheritance (Fesenko et al., 1998; Woo et al, 1999; and Zeller and Hasm, 2001) based on F_2 segregation in interspecific hybrids were not conclusive and the proposed genetic model was incomplete.

When wild species are used in breeding programs to improve cultivated species, both desirable and undesirable characters are introduced into interspecific hybrids. Seed shattering and winged seed characters from *F. homotropicum* occurred in the interspecific hybrids between *F. esculentum* and *F. homotropicum*. Seed shattering causes severe yield loss. Winged seed reduces seed density and causes problems for cleaning equipment. Therefore, both characters must be eliminated through efficient selection in breeding processes based on a solid knowledge of inheritance. However, genetic studies have not been conducted on either character in buckwheat. The inheritance of the winged seed character is of particular interest, as information cannot be based on studies in other crop species.

Based on this background information, this thesis has six objectives:

1. To study *F. homotropicum* based on intraspecific and interspecific crossability and cytological characterization to confirm the crossability of *F. homotropicum* diploid and tetraploid forms with the two cultivated species, and to explore the possibility of using *F. homotropicum* as a bridge species in hybridization of the two cultivated species, the origin of *F. homotropicum* tetraploid, and the relationships among *F. tataricum*, *F. homotropicum* and *F. esculentum*;

2. To conduct cytological analysis of the hybrids between *F. esculentum* and *F. homotropicum* (tetraploid) to provide useful information for buckwheat improvement;
3. To produce interspecific hybrids between *F. esculentum* and *F. tataricum* using different approaches to explore the possibility of producing viable and fertile hybrids for buckwheat improvement;

4-6. To determine the inheritance of self-compatibility, seed shattering and winged seed characters using intraspecific hybrids within *F. homotropicum* and interspecific hybrids between *F. esculentum* and *F. homotropicum* (diploid) to provide a genetic basis for efficient selection in buckwheat breeding.

2.0 LITERATURE REVIEW

2.1 General Review of Buckwheat

Buckwheat belongs to the genus *Fagopyrum* in the family *Polygonaceae*. *Fagopyrum esculentum* (common buckwheat) and *F. tataricum* (Tartary buckwheat) are the two cultivated species. Buckwheat grains, along with millet, sorghum and barley have been unearthed from a grave of the Western Han Dynasty (206 B.C. – 8 A. D.) in China (Li and Yang, 1992). Wang (1989) reported the similar archeological remains found in east Tibet, which can be dated to 2600 B.C. It is believed that buckwheat originated from the Southwest China; therefore buckwheat cultivation may start 5000 years ago (Ohnishi, 1998).

Based on isozyme analysis, there were two possible main distribution routes of common buckwheat and Tartary buckwheat from the center origin in the Southwest China to Asia, (Murai and Ohnishi, 1995). One route was from the Southern China through the Northern China to the Korean peninsula and into Japan; the other route was from the Southern China through Bhutan, Nepal, Kashmir of India, and further to Karakoram Hindukush of Northern Pakistan. Common buckwheat probably was introduced into Europe in the Middle Ages from Siberia, reaching Germany early in the 15th century and probably carried to North America by immigrants (Hughes and Hensen, 1934; Campbell, 1997; and Kreft, 2001).

2.1.1 Nutritional Values and Utilization of Buckwheat

The dehulled inner portion or groat of buckwheat seed is similar in structure to cereal grain, consisting of a starchy endosperm and an oily embryo and thus is often referred to as a dicot pseudo cereal (Tahir and Farooq, 1998). Buckwheat is one of the best sources of high quality proteins in the plant kingdom (Pomeranz, 1973) characterized by high content of albumins and globulins and low content of glutamic acid and proline. The

protein composition is high in arginine, aspartic acid and tryptophan and is particularly rich in lysine, a limiting amino acid in cereals (Campbell, 1997).

Buckwheat has significant potential applications in nutraceutical and pharmaceutical products and as a functional food because of its high nutrient value and medicinal qualities. Buckwheat is the best natural and the only edible plant source of rutin, a flavonol glucoside. Rutin's properties in human metabolism include increasing the elasticity of blood capillary tubes with associated reduction of hypertension and acting as an antioxidant of ascorbic acid used in the treatment of diabetes and cardiovascular diseases (Clemetson, 1976, 1979, Minami et al., 1998). Moreover, seeds of common buckwheat contain 1.5-3.7% total lipids. The high content of unsaturated fatty acids in groats or dehulled seeds contributes in the reduction of high blood pressure and in the prevention of arteriosclerosis (Belova et al. 1970, Campbell, 1997).

Buckwheat is utilized as human food in many different ways (Campbell, 1997). It is mainly consumed by humans as a grain crop, prepared as noodles, pancakes, biscuits, as a cereal and for bread, on an ingredient in making porridge and soup. It is a staple food in many mountainous areas in the Southeast Asia. Buckwheat is a traditional food in Asia and used as a component of holiday and religious celebrations in Japan, Nepal and Korea. In addition, buckwheat seed is fermented to produce alcoholic drinks, juice and vinegar. The flowers are a source of nectar for honey production. Buckwheat plants and leaves are consumed as a vegetable in many areas of the Indian subcontinent and are harvested several times in a year. Buckwheat is a useful green manure crop for the restoration of low-productivity land and as a smother crop for weed control. It is also used as a feed for livestock, poultry and as a food or a cover crop for wild life.

2.1.2 Buckwheat Production and Breeding

Buckwheat grows in the temperate regions of the world: China and Russia, the two largest buckwheat producers, followed by Ukraine, Kazakhstan, Poland, Brazil, USA, Canada and other small producers (Campbell, 1997; Wei, 1995; and Armstrong, 1999). Manitoba produces two-thirds of the buckwheat in Canada, and 70% of the crop is

exported to Japan (Armstrong, 1999). Buckwheat is a short season crop, maturing after two to three months of growth, thus its production fits with other crops such as cereals or potato in a crop rotation (Armstrong, 1999). For example, in Ukraine, buckwheat is sown in July after summer crops such as maize, barley, and wheat are harvested or before rice is planted (Alekseyeva, et al., 2001). In Canada, buckwheat is an ideal crop to grow in rotation with cereal crops. In many areas of the world, buckwheat is grown in the alpine mountainous region or under poor and risk production conditions because of its ability to achieve moderate yields on poor soils. Buckwheat is also more tolerant of acid soils and has a higher resistance to pest damage than other crops (Haque, 1995 and Choi et al., 1995).

Over the past several decades, germplasm of buckwheat (varieties, local landraces, mutants, and wild species) has been collected, evaluated, and preserved in many countries including China, Russia, India, Nepal, Korea, Japan, and Canada (Campbell, 1997). These provide a valuable genetic resource for buckwheat improvement.

Buckwheat improvement programs have achieved success in the past. With the exception of yield, which has been a priority in all crop improvement programs, breeding programs have varied their emphasis for such characteristics as increased seed size and frost tolerance, determinate growth habit, reduced seed shattering, greater lodging resistance, and higher rutin content. Many new varieties have been developed using recurrent selection and progeny evaluation, selection directly from varieties and landraces, production of artificial autotetraploid with four copies of one genome, and mutation breeding (Wei, 1995; Campbell, 1997; Fesenko, 2001; Fesenko, A.N., et al., 2001; Minami et al., 2001; Moiseenko et al., 2001; Morishita, et al., 2001; Suzuki, et al., 2001; Tang et al., 2001).

2.2 Phylogenic Studies in *Fagopyrum*

2.2.1 Classification of *Fagopyrum*

Fagopyrum contains 19 species that are divided into two monophyletic groups, the cymosum group and the urophyllum group (Ohsako et al., 2002). The cymosum group

consists of species with large, lusterless achenes incompletely covered by persistent perianths and with laterally long cotyledons. This group includes two cultivated species and two wild species. The urophyllum group consists of species with small, lustrous achenes completely covered with persistent perianths and with round or vertically long cotyledons. All species in this group are wild species. Phylogenetic analysis indicated that the two groups are distantly related to each other based on their morphology, isozyme variability, chloroplast DNA (cpDNA) variability, gene sequences of rbcL-accD of cpDNA, and the internal transcribed spacer (ITS) region in the nuclear ribosomal RNA (rRNA) (Ohnishi and Matsuoka 1996; Yasui and Ohnishi 1996, 1998a, and 1998b). Three clades are found in the cymosum group, the *F. esculentum-F. homotropicum* clade, the *F. tataricum* clade, and the *F. cymosum* clade (Yasui and Ohnishi, 1998b). In other reports, this group contained two clades, the *F. esculentum-F. homotropicum* and the *F. tataricum* - *F. cymosum* due to the close relationship between *F. tataricum* and *F. cymosum* (Ohnishi and Matsuoka, 1996).

2.2.2 Methods of Phylogenetic Study

Most of phylogenetic studies conducted in *Fagopyrum* have followed cladistic analyses. The methodology has been well developed in recent decades. Studies are no longer solely dependent on traditional morphological description, but instead on the combination of evidence from morphological, biological, cytological, and molecular analysis assisted by advanced techniques to analyze and interpret the relationship of organisms and to infer the evolutionary history.

Morphological study is still a useful tool for identifying species and as a supplemental method in phylogenetic analysis in *Fagopyrum.* Compared to traditional taxonomy, the current analysis for morphological characters is conducted in a more scientific and reliable manner. A group of characters is set up using data matrixes followed by precise algorithms and the construction of phylogenetic trees. The relationships of taxa are finalized by combing morphological data with other sets of data. Ohsako and Ohnishi (1998) used 14 morphological characters and isozyme analysis as well as crossability to identify 9 unknown collections from 9 suspected species. Based on the evidence, they

named two new species, *F. macrocarpum* and *F. rubifolium*. Ohnishi and Matsuoka (1996) constructed three independent phylogenetic trees based on morphology, isozymes and cpDNA variability. The three trees revised the classification of *Fagopyrum* by Steward (1930), showing good agreement and reflecting the phylogenetic relationships among the *Fagopyrum* species.

Crossability is often used in phylogenetic studies in *Fagopyrum*. Successful crosses between species or accessions imply close relationships between them (Ohsako and Ohnishi, 1998). In most studies, observations include the percentage of seed set (crossability) from a cross, the percentage of seed germination, and hybrid fertility. If the hybrid is sterile, sib-crossing or backcrossing with both parents are used to observe fertility restoration. Fertile hybrids indicate a very close relationship between two species. Examples of this include *F. esculentum* and *F. homotropicum* (Hirose et al., 1995), *F. statice* and *F. leptopodum*, as well as *F. macrocarpum* and *F. pleioramosum* (Ohsako et al., 2002). If crossability is high and the hybrid seeds easily germinate but the hybrid itself is sterile, this also implies a close relationship between the two parents (Ohsako and Ohnishi, 1998).

Cytological study in *Fagopyrum* is mostly used for determining the ploidy level based on mitotic chromosomes. Chromosome pairing at meiosis has been observed by Chen (1998) in interspecific hybrids. Genome *in situ* hybridization between *F. esculentum* ssp. *ancestrale* and *F. homotropicum* has been conducted by Asano (1998) without success due to the small differences between genomes of the two species. Chromosome banding in phylogenetic studies is limited due to the technical difficulties caused by the small chromosomes in *Fagopyrum*.

Molecular analysis has achieved the most success in phylogenetic studies of *Fagopyrum*. The studies include isozyme variability (Ohnishi, 1998b; Ohsako and Ohnishi, 1998; Asano, 1998), RADP makers (Murai and Ohnishi, 1996), cpDNA variability (Ohnishi and Matsuoka, 1996), rbcL and accD genes and their intergenic region in cpDNA (Yasui and Ohnishi, 1996 and Ohsako et al., 2001), the trnK gene intron in cpDNA (Ohsako et

al., 2002), and ITS region of the nuclear rRNA gene (Yasui and Ohnishi, 1998b; Ohsako and Ohnishi, 1998).

Parsimony and neighbor-joining analysis are two common methods that determine the phylogenetic relationships in the cladistic analysis of *Fagopyrum*. Bootstrap analysis and a heuristic search are often used to evaluate and support phylogenetic trees.

2.2.3 Progress of Phylogenetic Studies in *Fagopyrum*

The most important progress in the phylogenetic study of *Fagopyrum* is that a set of well-developed research methods has been gradually established over the last two decades. Another significant development is the identification and collection of new species. Since Ohnishi started collecting wild buckwheat in the Southwest China in 1988 (Ohnishi, 1995), eight new species and one new subspecies have been added to *Fagopyrum* (Ohsako et al., 2002). These include *F. homotropicum*, *F. capillatum*, *F. pleioramosum*, *F. callianthum* (Ohnishi 1998a), *F. rubifolium*, *F. macrocarpum* (Ohsako and Ohnishi, 1998), *F. esculentum* ssp. *ancestrale* (Ohnishi, 1998a and b), *F. gracilipoides* and *F. jinshaense* (Ohsako et al., 2002). *Fagopyrum homotropicum* has played an important role in common buckwheat improvement using interspecific hybridization due to its close relationship to common buckwheat.

The origin of common buckwheat had been previously suggested to be in Siberia or in the area of the Amur Reviver (De Candolle 1883). De Candolle (1883) believed that the origin of Tartary was in China, and Deng (1929) later proposed its origin to be in the Southwest China. The center of genetic diversity of the *Fagopyrum* species has been suggested in the Himalayas, Western Tibet (Komarov 1938, Stoletova 1958, and Krotov 1960) or in Southern China (Nakao 1957). Previously, *F. cymosum*, a perennial wild buckwheat, was considered as the putative ancestor of both common and Tartary buckwheat due to the morphological similarity (Gross, 1915; Steward, 1930; and Hedberg, 1945). After a three-year search for wild *Fagopyrum* species in China and the Himalayan hills, Ohnishi (1991) found *F. esculentum* ssp *ancestrale* and officially named it. Based on phylogenetic study, it has now been clarified that the subspecies *F.*

esculentum ssp *ancestrale* and *F. tataricum* ssp. *potanini* are ancestors of the two cultivated species, *F. esculentum* and *F. tataricum*, respectively (Ohnishi and Matsuoka, 1996 and Ohnishi, 1998a). The two ancestors differ from the two cultivated species only in dormancy and shattering habit which are the key characters distinguishing cultivated species from their wild relatives. It was also confirmed that the Southwest China (western Sichuan and northern Yunnan) is the center of genetic diversity of the *Fagopyrum* species (Ohinish, 1998a).

Phylogenetic studies have clarified the relationships between three species in the cymosum group. It had been thought that *F. cymosum* was more closely related to *F. esculentum* than to *F. tataricum* (Steward, 1930). However, Kishima et al. (1995) first pointed out that *F. cymosum* is much more closely related to *F. tataricum* than to *F. esculentum* based on RFLPs of cpDNA. This was confirmed by Ohnishi and Matsuoka (1996) based on isozyme and cpDNA variability and by Yasui and Ohnishi (1996) based on rbcL gene of cpDNA. It is difficult to separate the *F. tataricum* – *F. cymosum* clade from the *F. esculentum* – *F. homotropicum* on the basis of morphology, but they were clearly resolved using isozyme analysis (Ohnishi and Matsuoka, 1996).

Evolutionary changes in *Fagopyrum* are studied using the indicators of changes in floral morphology from distyly to homostyly, in mating systems from self-incompatibility to self-compatibility, in growth habit from perennial to annual, and in ploidy level from diploid to tetraploid (Yasui et al., 1998). The ancestor of buckwheat is assumed to be a distylic, perennial, and diploid species with self-incompatibility i.e. the inability of a fertile hermaphrodite seed-plant to produce zygotes after self-pollination (Nettancourt, 1977). Since three homostyle and self-compatible species (*F. tataricum* and *F. homotropicum* in the cymosum group and *F. gracilipes* in the urophyllum group) are included in different clades, breakdown distyly and self-incompatibility might have occurred independently several times. Subsequent reproductive isolation after self-incompatibility breakdown has played an important role for speciation in *Fagopyrum*. The breakdown of distyly and self-incompatibility has occurred relatively recently. This is because, in phylogenetic trees, no large group of species is entirely self-fertilizing and

for every self-fertilizing species, there is a closely related distylic species (Ohnishi and Matsuoka, 1996).

2.3 Characteristics of *Fagopyrum esculentum*, *F. tataricum*, and *F. homotropicum*

2.3.1 Two Cultivated Species – *F. esculentum* and *F. tataricum*

Common buckwheat (*F. esculentum*) is widely cultivated in the northern hemisphere and to a lesser extent in the southern hemisphere. Most breeding programs and international trade has focused on common buckwheat. The other cultivated species, Tartary buckwheat (*F. tataricum*) has had relatively little breeding effort. Its production is limited to the high altitude areas of Asia with the crop mainly being consumed locally (Campbell, 1997). However, production of common buckwheat is declining in many areas due to its low productivity and is replaced with finger millet (*Eleusine coracana*). or other crops, while Tartary buckwheat production stays constant (Campbell, 1997).

The low productivity of common buckwheat is primarily caused by low seed set (Adachi, 1990). Common buckwheat is a cross-pollinated species characterized by distylic flowers. The pin flower has long styles and short stamens, while the thrum flower has short styles and long stamens. The two types of flowers grow on separate plants. Fertilization can only occur between the pin and thrum flowers and is mainly accomplished by insects and wind. Common buckwheat flowers secrete nectar only in the morning and early afternoon, while bees prefer to work on the same crop plant all day, therefore bees prefer to work other plants (Campbell, 1997). A low presence of pollinators and inefficient pollination leads to poor fruiting in both types of flowers (Namai, 1991 and Fesenko N. V., 1990).

It was reported that moisture and temperature stress cause flower abortion in common buckwheat with incomplete development of the embryo sac and seed collapse in the post-zygotic stage, such that only approximately 12% of the flowers produce mature seeds (Tahir and Farooq, 1988; Adachi, 1990; and Campbell, 1997).

Damage from early spring and late fall frost on common buckwheat is a critical factor influencing grower preferences in many marginal production areas. Common buckwheat is very sensitive to freezing and experiments have shown that the crop was injured at temperatures of -2°C to -3°C (Gaberscik, et al., 1986). There is no frost tolerance available within the germplasm of the species and limited progress has been made in the breeding for frost tolerance, due to the difficulty in evaluation and the complex inheritance of the character.

The objective of improving seed set has been approached by the selection of self-compatible homostyle flowers produced by mutations in common buckwheat (Marshall, 1969; Nuszkowski, 1980; and Jablonski et al., 1986). However, there has been limited success, probably due to inbreeding depression, as a result of the accumulation of deleterious recessive alleles in the normally cross-pollinated populations (Kreft, 1983 and Williams, 1987).

Tartary buckwheat can be a source of beneficial characteristics, incorporated into common buckwheat through interspecific hybridization. These characters include self-compatibility, frost tolerance, high productivity, and high rutin content (Morris, 1951; Samimy, et al., 1996; Campbell, 1997, and Wang and Campbell, 1998). However, the interspecific cross has proven to be very difficult with few hybrids being produced and unrestored hybrid sterility (Samimy, et al., 1996 and Wang and Campbell, 1998).

2.3.2 Discovery of *F. homotropicum*

Fagopyrum homotropicum is a wild species found in the Southwest China, (Yunan and Sichuan provinces) and described by Ohinish (1995). Of the 19 species in the genus *Fagopyrum*, *F. homotropicum* is one of three species that is self-compatible with homostyle flowers. Phylogenetic studies showed that *F. homotropicum* is the most closely related species to common buckwheat and it has the same breeding system as another cultivated species Tartary buckwheat. It is believed that heterostyly with obligate outcrossing evolved to homostyly, allowing self-pollination to arise relatively recently. The progenitor of *F. homotropicum*, however, remains unknown (Ohnishi, 1995). It is not

clear whether the ancestor of *F. homotropicum* was from extant taxa or a more ancient origin from an extinct ancestor.

2.3.2.1 Genetic diversity of *F. homotropicum*

Some accessions of *F. homotropicum* are morphologically similar to the ancestor of common buckwheat (*F. esculentum* ssp. *ancestrale*) while plants from other localities seem to be more primitive (Ohnishi, 1995). Nineteen populations of *F. homotropicum* have been collected and studied, using morphological, cytological, and molecular analysis (Asano, 1998). Three populations are tetraploid (2n = 4x = 32) and the remaining populations are diploid (2n = 2x = 16). Isozyme analysis revealed that there is great diversity in the diploid populations, while the tetraploid populations have less variation among and within populations.

2.3.2.2 Origin of allotetraploid *F. homotropicum*

Based on isozyme analysis, Ohnishi and Asano (1999) suggested that *F. homotropicum* with 32 chromosomes is an allotetraploid, which contains two different genomes derived from two species. Heterozygosity at homologous loci in the three tetraploid populations is fixed at four out of 16 loci with disomic or diploid-like inheritance from generation to generation. Multivalents or univalents at meiosis are cytological evidence supporting autotetraploidy, but this is not definitive because selection for fertility could be effective in establishing bivalent pairing. For example, induced autotetraploid in maize (Singh, 1993) and buckwheat (Adachi et al., 1983) showed predominantly bivalent pairing at meiosis after repeated selection of individuals with high fertility. However, even though autotetraploid might form only bivalents, two of the four homologous chromosomes pair randomly, resulting in polysomic inheritance that does not follow normal Mendelian segregation laws. Therefore disomic inheritance that results from diploid-like pairing is evidence of an allotetraploid origin (Hancock, 1992 and Asano, 1998).

Allotetraploids exhibit bigenic disomic inheritance because they possess two divergent genomes from their diploid parents. Ohnishi and Asano (1999) suggested that *F. esculentum* spp. *ancestrale* might be one of the progenitors of the *F. homotropicum*

tetraploid, as all tetraploid populations shared an allele at PGM-1 with *F. esculentum* ssp. *ancestrale*, but this allele was not found in the diploid populations of *F. homotropicum*. Another possible progenitor could be a diploid accession, a Lijiang population of *F. homotropicum*, based on the phylogenic tree constructed by isozyme patterns (Asano, 1998). This population shared an allele at each monomorphic locus with the tetraploid *F. homotropicum*. One of the four loci with fixed heterozygosity in the tetraploid was also found in the Lijiang population.

2.3.2.3 Significance of *F. homotropicum* in buckwheat breeding

Buckwheat production has as long history as cereal crops. However, while wheat, maize, and other cereals are the predominant crops worldwide, buckwheat production has declined in many areas in the world (Campbell, 1997). The lack of necessary genetic variation for crop improvement is one of the main reasons for this decline. No frost tolerant germplasm has been found in common buckwheat accessions. Attempts to select for a self-compatible type in common buckwheat have made little progress. The introduction of self-compatibility from Tartary has not been successful due mainly to hybrid sterility.

The discovery of *F. homotropicum* has provided access to a new genetic resource that can be used to improve common buckwheat. The desirable characters in *F. homotropicum* include self-compatibility, frost tolerance, high level of seed set, green testa, and high rutin content (Campbell, 1997; Wang and Campbell, 1998). Successful interspecific hybridization is possible because *F. homotropicum* is closely related to common buckwheat (Ohnishi, 1995) and the technique of ovule rescue is now available (Suvorova, et al., 1994). A fertile hybrid plant between common buckwheat and *F. homotropicum* diploid was produced by Campbell (1995), using the ovule rescue technique. The hybrid provided the bridge to introduce self-compatibility from *F. homotropicum* into common buckwheat as well as improved yield potential in the backcross progeny.

2.4 Interspecific Hybridization

2.4.1 Interspecific Hybridization in Crop Improvement

Interspecific hybridization is a significant technique in crop improvement for the following reasons: the transfer of one or a few genes between species, the expression of new genetic recombinants, the production of new allopolyploid species that contain distinct genomes from different species, the production of doubled haploids, the creation of cytoplasm male sterility for use in hybrid production, and the construction of genetic linkage maps (Briggs and Knowles, 1967; Singh, 1993; Joobeur, et al., 1998, Messmer, et al., 1999; and Yasui, et al., 2001).

Interspecific hybridization is particularly useful when genetic variability is not available in the cultivated species (Hadley and Openshaw, 1980), for example, the transfer of disease resistance from wild species into cultivated species. Amphiploids produced through interspecific hybridization followed by chromosome doubling have been used successfully in plant breeding. The classic example is the creation of triticale produced by interspecific hybridization between wheat and rye (Morris and Sears, 1967 and Simmonds, 1979). Doubled haploid lines have been produced by genome elimination after interspecific or intergeneric hybridization in economically important crops such as barley and wheat, as an alternative to the anther or microspore culture technique (Pierik, 1987). Transferring the nuclear genome of one species into an alien cytoplasm can result in the expression of cytoplasmic male sterility. This method has been successfully applied in wheat, maize, and sorghum (Singh, 1993). For example, cytoplasmic male sterility in sorghum was obtained by transferring the kafir chromosome into the cytoplasm of milo (Poehlman and Sleper, 1995). Numerous genetic linkage maps have been constructed using segregating populations of interspecific hybrids. For example, genetic linkage maps of *F. esculentum* and *F. homotropicum* were constructed by AFLP (amplified fragment length polymorphisms) markers using a F_2 population of an interspecific hybrid between *F. esculentum* and *F. homotropicum* (Yasui et al., 2001).

2.4.2 Barriers to Interspecific Hybridization

2.4.2.1 External and internal barriers

Reproductive isolation is an important mechanism in speciation as it sets up the species' boundaries. This mechanism, however, becomes a barrier for successful interspecific hybridization in plant breeding. The mechanisms of reproductive isolation can be divided into external and internal barriers, or physical and genetic barriers (Bates and De Yoe, 1973 and Grant, 1981). External barriers separate species geographically such as mountains and rivers, ecologically such as growing in water or on land, temporally such as flowering in different seasons or times of the day, and mechanically such as avoiding outcrossing by hidden reproductive organs. This type of barriers is easily removed when interspecific hybridization is used in breeding programs, for example, manipulating planting time or storing pollen to coordinate flowering, or emasculating flowers to remove mechanical barriers.

Internal barriers to interspecific incompatibility include pre-zygotic (pre-fertilization) and post-zygotic (post-fertilization) barriers that prevent the development of a viable hybrid embryo (Grant, 1981 and Williams, 1987). In pre-zygotic barriers, pollen might fail to germinate, or the pollen is able to germinate but fails to penetrate the stigma, or the pollen is successful in penetrating the stigma but grows abnormally. Post-zygotic barriers result in embryo abortion due to zygotic or early embryonic inviability, or the disturbance of the normal embryo-endosperm-maternal tissue nutritional balance within the ovule, leading to the endosperm failing to develop or hypertrophy of maternal tissues at the expense of the embryos (Williams, 1987).

The mechanisms of interspecific incompatibility may be under genetic control in some plant species. The S-locus in self-incompatible species prevents both self-fertilization within a species and cross-fertilization among species (Nettancourt, 1977 and Hadley and Openshaw, 1980). Interspecific incompatibility can also be controlled by genetic systems independently from self-incompatible genetic systems (Nettancourt, 1977 and Hadley and Openshaw, 1980). It has been reported that two genes control crossability in the cross between tritordeum and triticale (Lima-Brito and Guedes-Pinto, 1998). It is also reported

that hexaploid and tetraploid wheat have different genetic systems that control crossability between wheat and rye (Liu, et al., 1999). Crossability of hexaploid wheat with rye is controlled by four loci located on chromosome 5B, 5A, 5D, and 1A, while the high crossability of tetraploid wheat with rye is controlled by three recessive alleles on chromosomes 1A, 6A, and 7A.

It is generally recognized that ploidy barriers cause interspecific incompatibility due to different ploidy levels, for example diploid and tetraploid, or different basic chromosome numbers of the genomes, for example x = 8, 9, and 10, or significant genetic distance between the genomes of the parents (Jackson and Hanneman, 1999). Failure of chromosome pairing can occur unequal chromosome numbers of the parents or lack of homology of chromosomes because of distant parents. Interspecific incompatibility can occur between the genomes of the parents, between the genome of one species and the cytoplasm of the other, or between the genotype of the zygote and the genotype of endosperm (Hadley and Openshaw, 1980 and Jackson and Hanneman 1999).

2.4.2.2 Hybrid sterility and hybrid breakdown

Hybrid sterility can occur following interspecific hybridization. Hybrids can be completely fertile, partially fertile, or completely sterile. The sterility of F_1 hybrids can be caused by chromosomal, genic, and cytoplasmic factors. Chromosomal hybrid sterility is caused by meiotic abnormalities resulting from failure of chromosome pairing (Williams, 1987). Genic hybrid sterility is defined as the occurrence of complete chromosome pairing with hybrid sterility. This is also called cryptic structural hybridity because the structural differences (translocation, inversion) between the corresponding chromosomes are too small to affect pairing (Singh, 1993 and Grant, 1981). The success of allopolyploid in evolution is dependent on the suppression of homoeologous pairing to ensure bivalent pairing only between homologous chromosomes. However, homoeologous pairing or intergenomic pairing can occur in interspecific hybrids when homologous chromosomes are not present, resulting in sterility from multivalent formation (Simmonds, 1979). Cytoplasmic hybrid sterility is expressed as male sterility

due to an imbalance between the nucleus and the cytoplasm (Petrova, et al., 1999 and Khrustaleva and Kik, 2000).

Hybrid fertility can be improved in successive generations, but fertility might also be reduced in F_2 plants from a F_1 fertile hybrid or the F_2 plants may become weaker than the parents and the F_1 hybrids (Williams, 1987 and Hadley and Openshaw, 1980). This phenomenon is referred as hybrid breakdown. In some cases, unfavorable gene combinations are not formed until the F_2 generation when more complete genetic recombination occurs (Hancock, 1992). Two explanations are provided for this situation (Hadley and Openshaw, 1980). Recombination of chromosome segments during meiosis in the F_1 hybrid that involves in small structural differences can lead to gametes with small, but significant deficiencies and duplications which may render the gametes inviable. The second explanation assumes complementary genetic systems. For example, the adapted genotype AABB has been selected for in the one species and the aabb genotype in the other species during evolution. When the two species are hybridized, the F_2 produces a few adapted genotypes of AABB or aabb but also produces many other genetic recombinants that lack adaptation.

2.4.3 Overcoming Interspecific Incompatibility

There are several ways to overcome interspecific incompatibility, including manipulation of crosses, production of bridge crosses, and embryo culture.

2.4.3.1 Manipulation of crosses

The success of the cross may depend on the genotypes of the parents and the direction of the cross. There is less success when the species with self-incompatibility is crossed with the species with self-compatibility compared to the reciprocal cross. This is called unilateral incompatibility, as the self-incompatible species rejects pollen from the self-compatible species, and is common in many plants (Nettancourt, 1977; Doganlar, et al., 1997; Chrungu, et, al., 1999; and Fesenko, et al., 1998). When two species with different ploidy level are crossed, for example, a diploid and a tetraploid, the parent with the higher chromosome number is generally used as the female to balance the

chromosome number in the endosperm (Allard, 1960 and Bohorva and Atanassov, 1990). Reciprocal differences and genotype specificity have been demonstrated in interspecific crosses in many plants (Hadley and Openshaw, 1980). It is recommend that the parental ploidy level is manipulated prior to crossing. For example, the chromosome numbers of the diploid parent can be doubled by colchicine treatment prior to crossing with another tetraploid parent (Hadley and Openshaw, 1980 and Poehlman and Sleper, 1995).

Stimulation of pollen germination and growth by regulators has been used to overcome interspecific incompatibility such as in wheat, rice, and cotton (Sitch and Snape, 1987; Altman, et al., 1987; and Mariam, et al., 1996). Other applications to increase the fertilization success include dead or fresh pollen from the female parent as a mentor mixed with male pollen or trimming of female styles to remove the stigmatic surface (Hadley and Openshaw, 1980).

2.4.3.2 Bridge crosses

Bridge crosses are an option to overcome the reproductive barriers between two distantly related species that are not crossable in spite of the skillful use of available techniques. This strategy has been practiced in plant breeding programs for Brassicas, tobacco (*Nicotiana tabacum*), cotton (*Gossypium hirsutum*), and potato (*Solanum tuberosum*) (Hadley and Openshaw, 1980; Vyas et al., 1995; and Vroh Bi et al. 1999). This technique uses one or two bridge species to connect two targeted species that cannot be hybridized directly through three way, four way, and even multiple crosses. For example, *Brassica rapa* as a bridge species was crossable with the wild species *Diplotaxis siettiana.* Three way crosses were made of (*D. siettiana* x *B. rapa*) x *B. juncea* to produce the hybrid between *D. siettiana* and *B. juncea* or the cross (*D. siettiana* x *B. rapa*) x *B. oleracea* to produce the hybrid between *D. siettiana* and *B. oleracea* (Vyas et al., 1995). Hybridization through bridge crosses is time consuming as it involves several cycles of crossing.

Somatic hybridization has also been used to overcome interspecific incompatibility between distant species when sexual hybridization has proven impossible. In this

technique, two parental protoplasts, obtained from enzymatic digestion of the somatic cell wall, are fused to produce hybrids. Those hybrids have combined chromosome numbers and cytoplasm from both parents (Pierik, 1987).

2.4.3.3 Embryo, ovule and ovary rescue

Embryo culture *in vitro* has been an efficient technique to overcome post-zygotic barriers to prevent embryo abortion. Many interspecific hybrids have been developed using this technique. Embryos can be cultured directly when they are large enough to be dissected from the ovules prior to seed abortion. Ovules containing embryos or even ovaries containing ovules can be cultured when the embryos are too small to be dissected from ovules at the time of seed abortion (Sharma, et al., 1996). Two critical factors determine the success of embryo development: the stage of embryo at the time of dissection and the composition of the medium (Pierik, 1987). Younger embryos require a more complex medium and the chance of success is lower. Abortion can occur in very young embryos (Hoch et al., 1995). Two approaches can be used to improve the efficiency of culturing young embryos (Hadley and Openshaw, 1980 and Pierik, 1987). One method is the application of growth regulators after pollination to maintain the embryos *in vivo* long enough to reach the critical size for culturing. Another approach is the improvement of the medium to satisfy the nutritional requirements for young embryo development. The medium modifications include the increased concentration of sucrose to maintain high osmotic pressure and the use of growth regulators, extracts, coconut milk, amino acids, and casein hydrolysate to stimulate embryo growth (Narayanaswami and Norstog, 1964; Pierik, 1987; and Hoch et al., 1995).

2.4.4 Chromosome Doubling

The most common methods used to restore hybrid fertility are chromosome doubling and backcrossing. Fertility is restore by chromosomal doubling if the sterility is caused by chromosomal instability. If the sterility is genic or genetic, backcrossing is the only option in restoring fertility (Hadley and Openshaw, 1980). Cytoplasmic hybrid sterility has applications in the production of hybrid cultivars. This form of sterility is under

genetic control and has to be restored by crossing with a line that has a nuclear restorer gene(s).

Colchicine is the most common chemical used for chromosome doubling. It disrupts mitosis by blocking the formation of the microtubules and preventing the polar migration of chromosomes, resulting in a cell with a doubled chromosome number (Levan, 1939). The efficiency of application is primarily affected by concentration, duration of the treatment, the application method used, and stages of plant materials (Hadley and Openshaw, 1980).

2.4.4.1 Allopolyploid and autopolyploid

Allopolyploid and autopolyploid are the two basic polyploids in plants (Singh, 1993). Allopolyploids originate from the doubling of the chromosome sets from two or more than two different species. Since each chromosome is represented twice, the chromosomes form normal bivalents at meiosis, referred to as diploid-like pairing, resulting in the formation of normal gametes. Autopolyploids contain more than two sets of the same chromosome complement or monoploid chromosomes sets. In general, autopolyploids are morphologically larger than the diploid in the size of the plants, leaves, and seeds, and have thicker and darker leaves, owing to the increased size of the nuclei. Since each chromosome has more than one pairing partner, higher orders of pairing are possible, producing multivalents at meiosis. The multivalent formation results in abnormal segregation and unbalanced gametes, reducing fertility with the production of aneuploid progeny, such as trisomic (2n+1) or monosomic (2n-1). The segregation of autopolyploids shows polysomic inheritance rather than disomic inheritance.

2.4.4.2 Amphiploid

Two distantly related species with distinct genomes can be crossed to produce a fertile amphiploid if the sterile F_1 hybrid is treated with colchicine to double the chromosome number. Homologous chromosome pairing leads in exclusively bivalent formation. For example, triticale (hexaploid AABBRR or octoploid AABBDDRR) was produced from the interspecific cross between tetraploid (AABB) or hexaploid (AABBDD) wheat and

diploid (RR) rye following by chromosome doubling of the F_1 hybrids (Simmonds, 1979).

Amphiploid hybrids can also be obtained by crossing two induced autotetraploids (Williams, 1987). For example, tetraploid trihordeum (2n = 4x = $DDH^{ch}H^{ch}$) was produced from the cross *Triticum tauschii* (DDDD) x *Hordeum chilense* ($H^{ch}H^{ch}H^{ch}H^{ch}$). Tetraploid triticale (2n = 4x = DDRR) was produced from *T. tauschii* (DDDD) x *Secale cereale* (RRRR) (Martin, et al., 1999). In addition, chromosomes doubling can occur spontaneously in the regenerants of F_1 hybrids during *in vitro* culture (Dahleen and Joppa, 1992).

2.4.4.3 Autoallopolyploid

An autoallopolyploid can be produced through chromosome doubling of the F_1 hybrid in some interspecific crosses. For example, an allotetraploid AABB is crossed with a closely related diploid or backcrossed with one of its ancestral diploids, for example A'A'. The F_1 will be a triploid AA'B. An autoalloploid AAA'A'BB will be produced after the chromosomes of the F_1 hybrid are doubled. The three genomes of this hexaploid are largely but not completely homologous with each other (Stebbins, 1971). This autoallohexaploid (AAA'A'BB) would differ somewhat in cytological behavior and fertility from an allohexaploid (AABBCC) that is derived from a triploid hybrid ABC (Hadley and Openshaw, 1980). The allohexaploid has normal diploid-like chromosome pairing at meiosis leading to the same normal growth and fertility as its respective diploids. However, the autoallohexaploid combines the characteristics of both an autopolyploid and an allopolyploid. The AAA'A'BB has normal homologous chromosome pairing in the genome BB and abnormal chromosome pairing in the AAA'A' genome, resulting in lower fertility than the respective diploids.

2.4.4.4 Unreduced gametes

During interspecific crossing, the chromosome complement can be doubled spontaneously at meiosis due to unreduced gametes. An amphiploid is formed from both unreduced female and male gametes (2n + 2n) and a triploid is formed from unreduced

gamete of either parent (n + 2n) or (2n + n) (Poehlman and Sleper, 1995). For example, in Brassicas, the hybrid *Enarthrocarpus lyratus* (2n = 2x = 20) x *B. carinata* (2n = 4x = 34) had 37 chromosomes instead of 27 chromosomes, as a result of fertilization of unreduced eggs (Gundimeda et al., 1992). In another report of an intergeneric cross in Brassicas (Vyas et al., 1995), two hybrids from the crosses *Diplotaxis erucoides* x *B. oleracea* and *D. erucoides* x *B. rapa* resulted from unreduced male gametes.

Interspecific hybrids frequently produce unreduced gametes because of the disturbances at meiosis that are associated with lack of chromosome pairing (Allard, 1960). For example, unreduced egg gametes occurred in the BC_1 and BC_2 generations in the cross between *Triticum aestivum* and *Agropyron cristatum* (Chen et al., 1992). The synthetic species *Raphnobrassica* was produced from the cross between cabbage *Brassica oleracea* (2n = 18) and radish *Raphanus sativus* (2n = 18) by Karpechenko in 1928 (Russell, 1998). The F_1 hybrids had 18 chromosomes and were mostly sterile due to failure of chromosome pairing at meiosis. However, a few seeds were produced and some of seeds were fertile. The resulted plants had 36 chromosomes that were the full diploid chromosome sets from both parents. This hybrid species was called *Raphnobrassica* combining the names from both parents.

2.4.5 Restoration of Hybrid Fertility by Backcrossing

In interspecific hybridization, backcrossing has been used to restore hybrid fertility when chromosome doubling is not an option for restoration of genetic or genic sterility. The recurrent parent (usually the cultivated species in a wide cross) can be directly backcrossed with the sterile F_1 hybrids or with the amphiploid hybrid after chromosome doubling. Fertility is usually restored after several successive backcrosses. The final products often revert back to the normal chromosome number of the recurrent parent, with the addition of the desirable gene or chromosome segment from the wild species as a result of genetic recombination during backcrossing. This may require selecting for the desirable traits to achieve introgression occurring.

A number of reports have demonstrated the importance of backcrossing in plant breeding programs using wide crosses. For example, the *Aegilops kotschyi* cytoplasm and a chromosomal segment carrying genes for resistance to leaf rust were transferred to common spring wheat cultivars by repeatedly backcrossing (Ikeguchi, et al., 1999). In the cross *Helianthus annuus* x *H. hirsutus*, backcrossing to the cultivated sunflower species restored the chromosome number of *H. annuus* after the fifth backcross (Bohorva and Atanassov, 1990). In cotton, amphihexaploids were produced from the cross between cultivated cotton *Gossypium hirsutum* (AADD) and the wild Australian *Gossypium* diploid species with one of C, G, and K genomes. The fertility was restored by repeatedly backcrossing the amphiploid with the cultivated cotton. The final products were tetraploid AADD plants carrying the recombinant chromosome fragments of the C, G, or K genomes (Brubaker, et al., 1999).

2.4.6 Interspecific Hybridization in Buckwheat

Attempts to produce interspecific hybrids in buckwheat were initiated in 1951 (Morris, 1951) but the first interspecific hybrid was not obtained until 1975 (Krotov and Dranenko, 1975). To date, interspecific hybrids used for buckwheat breeding have been developed among species *F. esculentum, F. tataricum, F. homotropicum,* and *F. cymosum*, all belonging to the cymosum group. The technique of *in vitro* ovule culture has played a very important role in the production of hybrids. Several researchers have also applied somatic hybridization between *F. esculentum* and *F. tataricum* without success (Lachmann, et al., 1994). Woo (1998) has isolated the protoplast of *F. esculentum* from the egg for *in vitro* hybridization.

2.4.6.1 Interspecific barriers

When a self-compatible species is crossed with a self-incompatible species, both unilateral compatibility (only crossable in one direction) and bilateral compatibility (crossable in both directions) can be expressed. There are examples of both types in *Fagopyrum*. The cross between Tartary and common buckwheat expresses unilateral incompatibility (Morris, 1951; Samimy, 1991; Hirose, et al., 1995; Chen, 1998; and Fesenko, I.N., et al, 2001), as the cross was successful only when the self-compatible

Tartary buckwheat was used as the female. In the cross between common buckwheat and *F. homotropicum*, Hirose et al. (1995) reported that the crosses showed unilateral incompatibility. However, Fesenko I.N. et al. (2001) concluded that the two species were bilaterally compatible with the observation that, when common buckwheat was used as the female, the thrum flowers were more compatible with *F. homotropicum* than the pin flowers.

The interspecific barriers in *Fagopyrum* include pre-zygotic, post-zygotic barriers and hybrid sterility. In the cross between Tartary buckwheat and common buckwheat, both pre-zygotic (Shaikh, et al., 2001) and post-zygotic barriers have been reported. The penetration of the pollen tube of Tartary was inhibited at the top of the styles of common buckwheat (Hirose, 1995 and Morris, 1951) or the pollen tube only reached the middle part of the style (Hirose, 1995). In reciprocal cross between Tartary and common buckwheat, the pollen tube of common buckwheat reached the base of the style and penetrated into the embryo sac with no fusion between the gametes (Samimy, 1991). If fertilization occurred, embryo growth reached only one-half the size of the mature seed before turning brown and aborting (Morris, 1951). In the cross between common buckwheat and *F. homotropicum*, fertilization occurred normally (Hirose, 1995), but the post-zygotic barrier had to be overcome mainly by ovule rescue *in vitro* (Campbell, 1995).

Among the four species that have been used in interspecific hybridization in *Fagopyrum*, it is apparent that the cross between *F. esculentum* and *F. homotropicum* is the easiest to achieve and the cross between the two cultivated species, common and Tartary buckwheat, is the most difficult (Hirose, 1995; Wang and Campbell, 1998; Shaikh, et al., 2001; and Fesenko, I. N. et al, 2001). Fesenko, I. N. et al. (2001) indicated that there might be a correspondence between the difficulty of the crosses and the relative difference in the chromosome sizes between species. For example, the average chromosome sizes of common buckwheat, *F. cymosum*, and Tartary range from the longest (3.78-6.97μm), intermediate (2.6-4.6μm), and the shortest (2.0-3.3μm) respectively. The genomes of common buckwheat and Tartary have the least congruence

compared to the genomes either between common buckwheat and *F. cymosum* or between *F. cymosum* and Tartary buckwheat. Nagano et al. (2000) reported that the average nuclear DNA amount of *F. esculentum* and *F. cymosum* were similar at 2.77 pg (1pg = 965 Mbp) and 2.32 pg, respectively, while *F. tataricum* was only at 1.11 pg. This agrees with the cytological observation.

Both backcrossing and chromosome doubling have been used to overcome F_1 hybrid sterility in buckwheat. For example, the sterile hybrids of *F. esculentum* x *F. cymosum* were backcrossed with *F. esculentum* repeatedly (Suvorova, 2001). An amphidiploid has been produced from the cross between induced tetraploid *F. tataricum* and the natural tetraploid *F. cymosum* (Krotov, and Dranenko, 1975). The fertility of the hybrid between *F. esculentum* (2x) x *F. homotropicum* (4x) has been restored by colchicine treatment of the F_1 hybrids (Wang, et al., 2001).

2.4.6.2 Status of interspecific hybridization in buckwheat

The first successful interspecific hybrid between Tartary buckwheat and *F. cymosum* was reported by Krotov and Dranenko (1975). Both parents were autotetraploid (2n = 4x = 32). The amphidiploid hybrid was named as *F. giganteum*. Subsequently, efforts have been made to use this synthetic species in the improvement of cultivated Tartary buckwheat by backcrossing (Fesenko, et al., 1998). Partially fertile progeny have been obtained from the BC_1F_3 generation that expressed the combined characteristics of both parents.

Nagatomo (1961) first attempted to carry out the cross between *F. esculentum* and *F. cymosum* without success. A concerted effort to improve *F. esculentum* by *F. cymosum* was made in the 1990s. The first successful sterile hybrid was reported by Ujihara et al. (1990) using the two tetraploid parents. Suvorova also developed sterile hybrids during this period (Suvorova et al., 1994) and subsequently attempted to restore hybrid fertility by backcrossing (Suvorova, 2001). These crosses have been repeated by Ujihara's laboratory in Japan (Hirose, et al, 1995), by Rumyantseva, et al. (1995) in Russia and the Ukraine, and in Campbell's laboratory in Canada (Woo, et al, 1999b) using both the

tetraploid and diploid parents. The common problem is the difficulty to completely restore hybrid fertility (Suvorova, 2001).

The second focus of interspecific hybridization efforts in the genus *Fagpyrum* was the cross between common buckwheat and the wild species *F. homotropicum* after *F. homotropicum* was first collected by Ohnishi (1995). The first hybrid was developed by Campbell (1995), as part of a breeding effort to produce new self-pollinated buckwheat with stable yield. Similar efforts are underway in buckwheat breeding programs in Russia, Japan, Korea, and Germany, as reported at the International Buckwheat Symposium held in Korea in 2001 (Fesenko, I. N., et al, 2001; Ohmoto, et al, 2001; Kim, S. K., et al., 2001, Kim, Y. B., et al, 2001; and Zeller and Hsam, 2001).

The cross between the two cultivated species, one expressing self-incompatibility and the other self-compatibility, has been the most challenging hybridization to date. Morris (1951) initiated this cross in an attempt to combine the self-compatibility from Tartary buckwheat and the larger seed size with looser seed coat from common buckwheat. The effort failed due to embryo abortion. As the technique of ovule culture was developed, some researchers repeated this cross at both the diploid and tetraploid level assisted by *in vitro* ovule rescue. These included Samimy (1991) and Samimy et al. (1996) from the USA, Fesenko, I. N. et al. (2001) from Russia, Chen (1998) from China, Wang and Campbell (1998) from Canada, and Hirose et al., (1995) and Woo (1998) from Japan. Samimy et al. (1996) successfully overcame interspecific incompatibility to develop a hybrid from the cross between common and Tartary buckwheat. They first developed a common buckwheat population with similar isozyme patterns to those found in Tartary buckwheat and then crossed this population to Tartary buckwheat. One hybrid was obtained after 263 ovules were cultured *in vitro*. The sterile hybrid was confirmed by isozyme analysis; however, fertility could not be restored. Wang and Campbell (1998) reported success in the same cross, when they obtained three sterile hybrids *in vitro* culture from 111 ovules.

In addition, several additional hybrids have also been reported from the cross between Tartary and *F. homotropicum* (Wang and Campbell, 1998) and the cross between *F. cymosum* and *F. homotropicum* (Woo, et al., 1999b).

Some researchers have developed protocols for protoplast isolation and regeneration from common buckwheat (Rumyantseva and Lozovaya, 1987; Adachi, et al., 1989; Gumerova, 1991) and Tartary (Lachmann and Adachi, 1990 and 1991; and Zhang et al., 1992). Somatic hybridization between common and Tartary buckwheat was carried out by Lachmann, et al. (1994), which resulted in the formation of callus cells containing recombinant DNA of the parents.

2.5 Inheritance of Self-incompatibility and Self-compatibility, Seed Shattering and Winged Seed

2.5.1 Inheritance of Self-incompatibility and Self-compatibility

Plant breeders have targeted the introduction of self-compatibility to improve yield stability in self-incompatible species. Self-compatibility can be produced in the following ways: 1) selecting natural mutants of the self-compatible form from self-incompatible populations as in buckwheat (Marshall, 1969; Fesenko and Antonov, 1973; Ruszkowski, 1980; and Jablonski, et al. 1986); 2) inducing self-compatible mutants as in fruit trees (Lapins, 1983) and in flowering plants of *Enothera*, *Prunus* and *Trifolium* (Lewis and Crowe, 1958 and Socias i Company, 1990); 3) inducing self-compatible autopolyploids from self-incompatible diploids as in common buckwheat (Adachi, et al, 1982) and potato (Nettancourt, 1977); and 4) transferring self-compatibility from self-compatible species into self-incompatible species as in buckwheat between *F. homotropicum* and *F. esculentum* (Campbell, 1995) and in fruit trees between peach and almond (Socias i Company, 1990). The most common method is the transfer of self-compatibility from one species into another species through interspecific hybridization. For this to be successful, it is very important to understand the inheritance of self-incompatibility and self-compatibility.

2.5.1.1 Distribution of self-incompatibility in the plant kingdom

Self-incompatibility, as an outbreeding mechanism, is common in the plant kingdom. It occurs in approximately 26 genera in the Angiosperms (Nettancourt, 1977), including such commercially important cultivated species as clover, alfalfa, cabbage, kale, sunflower, rye, Brassica, several grasses, sugar beet, diploid potato, cherry and olive.

Homostyly occurs in both self-pollinated species as in *F. homotropicum* and cross-pollinated species as in *Cardamine pratensis* (Nettancourt, 1977). However, heterostyly mostly occurs in cross-pollinated species in the form of distyly (pin and thrum), such as in *Primula*, *Pulmonaria* and *Fagopyrum*, and in the form of tristyly (long, middle and short style), as in *Lythrum* and *Oxalis* (Nettancourt, 1977). The exception is that self-incompatibility can be broken down in distyly when genome is duplicated from diploid to autotetraploid (Adachi et al., 1982 and Ohsako et al., 2002). Heterostyly has been documented in 24 families to date. Distyly is far more common than tristyly that was found in only three families (Judd, et al., 1999). In general, homostyle self-incompatibility occurs more frequently than heterostylic compatibility in cultivated species (Nettancourt, 1977). The buckwheat genus *Fagopyrum* includes three homostyle self-compatible species (diploid), two distylic self-compatible species (autotetraploid), and 14 distylic self-incompatible species (Ohsako, et al., 2002).

2.5.1.2 Gametophytic and sporophytic self-incompatibility

Gametophytic and sporophytic self-incompatibility are the two common classes of self-incompatibility in plants (Hancock, 1992). In gametophytic systems, pollen germination and tube growth depends on the genotype of the pollen itself. As an example a S_1S_2 stigma cannot be fertilized by S_1 or S_2 pollen, but can be fertilized by S_3 or S_4 pollen. In sporophytic systems, pollen performance is based on the genotype of the male parent. The pollen grain can germinate if it contains the same allele as the stigma, as long as the pollen parent has the distinct genotype from the female parent. For example, s pollen from ss plant is compatible with a Ss stigma.

Nettancourt (1977) has summarized studies on the inheritance of self-incompatibility in plants. In gametophytic homostyle system, self-incompatibility is controlled by a single gene locus with a polyallelic series. This is common in many species of Solanaceae and Leguminosaea. This type of system may also be controlled by two genes (S and Z) with a polyallelic series in the grasses, two genes (S and R) with an epistatic interaction in *Solana* species; and three to four genes in species of Anunculaceae and Chenopodiaceae. In sporophytic homostyle system, it has been postulated that self-incompatibility is controlled by two loci (T and S) with a two allelic epistatic interaction in *Capsella grandiflora*; two independent loci (B and G) with two alleles in *Carthamus flavescens*; and polyallelic control by a single locus in the case of both *Parthenium argentatum* and *Crepis foetida.*

Sporophytic heterostylic systems include both tristyly and distyly. In the tristylic system of *Lythrum* and *Oxalis*, two loci (S and M) with two alleles control self-incompatibility (Nettancourt, 1977). In distyly, it is widely accepted that a single genetic locus S with two alleles controls self-incompatibility. However, a supergene complex has been proposed in some studies (Lewis, 1954; Dowrick, 1956; and Sharma and Boyes, 1961).

Self-incompatibility in the genus *Fagopyrum* belongs to sporophytic distylic system that is controlled by a single genetic locus S (Althausen, 1908; Dahlgren, 1922; Eghis, 1925; Garber and Quisenberry, 1927; and Saknarov, 1946) the same as in *Primula* and *Pulmonaria* (Nettancourt, 1977). The genotype of the thrum phenotype is Ss and the pin genotype is ss. When the two types of flowers are cross-pollinated, the segregation of thrum and pin plants in the progeny will be in the ratio of 1:1. This mechanism is similar to the XY system of sex determination operating in animals and has similar consequences for outbreeding (Lewis, 1949).

A supergene complex that controls sporophytic self-incompatibility in distylic species has been suggested for *Jepsonia parryi* (Ornduff, 1970), *Primula viscose* (Ernst, 1936) and *Fagopyrum esculentum* (Sharma and Boyes, 1961). In this model, the supergene complex is made up of five subgenes that correspond to stylar incompatibility (I_s), pollen

incompatibility (I_p), stylar length (G), pollen size (P), and filament length (A) respectively (Lewis, 1954, Dowrick, 1956, Sharma and Boyes 1961). This model has been demonstrated to occur in buckwheat by Sharma and Boyes (1961), who produced mutants through X ray treatment on seeds. One of the mutant plants had both thrum and homostyle flowers. The homostyle flowers had the same self-incompatibility reaction as thrum flowers when they were selfed or crossed with pin flowers. This result infers that only one of the subgenes, the one that controlled the stylar length, had been changed. However, Sharma and Boyes (1961) also observed some thrum flowers after treatment had both self-compatible and self-incompatible reactions. In the same plant, thrum flowers set seeds when they were selfed but produced no seeds when they were crossed with other thrum plants and produced seeds when they were pollinated by other pin flowers. This result infers that the S locus may operate during selfing but not during crossing between thrum flowers. Therefore, the supergene model has not been finalized, as self-compatibility may be under different genetic control from self-incompatibility (Nettancourt 1977).

2.5.1.3 Inheritance of self-compatibility

Some genera contain both self-incompatible and self-compatible species, such as in the genera *Fagopyrum* and *Lycopersicum*. Inheritance of self-compatibility in these genera has been studied using self-compatible mutants that have arisen from self-incompatible populations or using interspecific hybrids between self-compatible and self-incompatible species.

Self-incompatibility has been established in the Angiosperms almost from the time of their origin (Whitehouse, 1950). Lewis and Crowe (1958) postulated that the self-compatible species in families with predominance of self-incompatibility have been derived by a degradation process (SI→Sc→Sc'→SC) of the alleles from their self-incompatible ancestors. Therefore, self-compatibility is controlled by a self-compatible allele, S_f, which is believed to be from the S series (Lewis and Crowe, 1954). Pandey (1968) pointed out that the S_f allele for self-compatibility might have been transferred

from a related species by interspecific hybridization, as the S locus seems to be allelic in closely related species.

However, some authors indicated that the breakdown of self-incompatibility is controlled by one or more major genes that are unrelated or not completely related to the S-locus. In *Chrysanthemum morifolium*, self-compatibility is associated with more than one major gene modified by other genes such as the S-genes (Ronald and Ascher, 1975). In *Primula sinensis*, the breakdown of heterostyly is due to two different independent loci having pleiotropic effects, rather than to mutation in the alleles at the S-locus (Beale 1939 and Mather, 1950).

Martin (1961, 1964, and 1968) has identified the presence of a dominant sporophytic switch allele necessary for the expression of self-incompatibility in the tomato species of *Lycopersicum*. The genotypes of the parents and progeny in his model is illustrated in the following:

1. SxSy AA represents the genotype of self-incompatible species.
   Sx and/or Sy control self-incompatibility and are activated by a dominant allele A. Self-compatible (Sc) pollen is rejected by Sx or/and Sy.
2. ScSc aa represents the genotype of self-compatible species
3. ScSx Aa or ScSy Aa is the genotype of the F_1 hybrids.
   F_1 is self-incompatible as Sx or Sy is activated by A and Sc pollen is rejected by Sx or Sy.

In F_2 progenies, any plant lacking either Sx or Sy combining with A will display a self-compatible phenotype.

In buckwheat, F_2 progeny from homostyle hybrids between *F. homotropicum* and *F. esculentum* expressed a 3:1 (homostyly: pin) ratio (Campbell, 1995; Wang and Campbell, 1998; Fesenko et al. 1998; Woo et al, 1999; and Zeller and Hsam, 2001), indicating that a single dominant gene might control self-compatibility. Woo et al. (1999a), proposed a single gene model with three alleles S> S^h >s to describe the relationships between the

three types of flowers related to self-compatibility and self-incompatibility. The genotype of the self-incompatible thrum flower is Ss or SS^h with thrum S being dominant to homostyly S^h and pin s. The genotype of the self-compatible homostyle flower was S^hS^h or S^hs with S^h being dominant to the s pin.

In the cross between *F. esculentum* thrum (Ss) and *F. homotropicum* (S^h S^h), the F_1 hybrids had a ratio of 1 homostyly (S^hs): 1 thrum (SS^h). The F_2 from the homostyle hybrid (S^hs) showed a ratio of 3 homostyly: 1 pin. These results support a one gene model. However, when Woo et al. (1999a) backcrossed the F_1 thrum hybrid SS^h with the pin (ss) of the *F. esculentum* parent, they obtained a segregation of 5 thrum: 5 homostyly: 1 pin in 11 plants. The pin plant was unexplained by the single gene model. Fesenko, et al. (1998) made the same cross as Woo, et al. (1999a) and observed the same results in the F_1 and F_2 from the homostyle hybrid. Instead of backcrossing the F_1 thrum hybrid with pin plants of *F. esculentum*, they allowed the hybrids to randomly mate with pin plants of *F. esculentum*. All three flower phenotypes, including pin, appeared in the resulting progeny. Therefore, Fesenko, et al. (1998) concluded that homostyly of *F. homotropicum* is not an allelic variation of the heterostylic gene of *F. esculentum*. Marshall (1969) also indicated that the form of self-compatibility selected from a self-incompatible population of *F. esculentum,* was influenced by at least two modifying genes that controlled the difference in the length of the style. Therefore, the inheritance of self-compatibility in buckwheat remains unresolved.

2.5.2 Inheritance of Seed Shattering

2.5.2.1 Seed shattering in wild and cultivated plants

Many wild plant species rely upon seed dispersal through a mechanism of shattering of the mature inflorescence, pods, seeds, or dehiscence of the pods. Non-shattering individuals that originated by spontaneous mutation are at a disadvantage for seed dispersal under natural conditions, as compared with individuals with a shattering habit (Ohnishi, 1999). However, the non-shattering variation was selected consciously or unconsciously by humans during the domestication of different crops (Cai and Morishima, 2000).

The shattering habit is an undesirable agronomic character, which occurs in many cultivated crop species, including the cereal crops of wheat, rice, and barley, the grain crops of sorghum and pearl millet, the oilseed crops of Brassicas, the legume crops of soybean, bean, lentil and pea, the forage crops of grasses, and the pseudo-cereal crop of buckwheat. The shattering habit not only seriously affects yield, but also affects the seed quality and viability due to the necessity of harvesting immature seeds to avoid severe grain loss (Young, 1986 and Burson, et al., 1978).

2.5.2.2 Factors that affect seed shattering

Many factors may affect shattering, including genetics, anatomic structures, and the environment. Seed shattering has been found to be related to the strength of the basal portion of the lemmas and glumes (Chang, 1943) and to the acute glume pair angle (Jones and Nielson, 1992) in wheat; the orientation of the cells in the pericarp in birdsfoot trefoil (*Lotus corniculatus*) (Grant, 1996); and the occurrence of an abscission layer in forage grasses (Burson, et al., 1978 and 1983 and Piccirilli and Falcinelli, 1989) and in buckwheat (Oba, 1998). Environmental factors, such as wind, rain, humidity and the time of harvest, all may contribute to yield loss due to shattering (Clarke, and Depauw, 1983 and Grant, 1996).

Crop management practices used to reduce yield loss from shattering include timely harvest, adjustment of the combine speed (Haffar, et al., 1991), and the application of chemicals such as phenolic acid and cobalt chloride to reduce shattering in soybean (Setia, et al., 1989 and Priti, et al., 1998). Breeding for shattering resistance has been attempted through recurrent selection in *Lotus* (Grant, 1996), by mutagenesis in *Papaver bracteatum* (Levy, 1985) and soybean (Rajput, and Sarwar, 1998), and by interspecific hybridization, as in *Brassica rapa* x *B. juncea* (Prakash, and Chopra, 1990). The use of interspecific hybridization in cultivar improvement has sometimes been restricted by the introduction of shattering from wild species into the cultivated species, such as occurred in the cross between common buckwheat and *F. homotropicum*. Reduction of seed shattering is therefore an objective in buckwheat breeding programs.

2.5.2.3 Inheritance of seed shattering

The inheritance of seed shattering has been studied in several ways, including the estimation of heritability by analysis of variance; by segregation patterns of the hybrids through intraspecific or interspecific hybridization; and by molecular analysis. Young (1991) estimated the narrow-sense heritability, using parent-offspring regression, in two populations of kleingrass (*Panicum coloratum*) and concluded that the inheritance of seed retention is low and the interactions of genotype x environment are a large component of the total variation. Hybridization between shattering and non-shattering genotypes has been a common method in the study of the inheritance of shattering, for example in rice (Woods, and Clark, 1976 and Elliott, et al., 1977) and in buckwheat (Fesenko, et al., 1998 and Ohnishi, 1999). Linkage maps constructed by molecular AFLP and RFLP (restriction fragment length polymorphism) markers has revealed the number of genes that control seed shattering in rice *Oryza stativa* x *O. rufipogon* (Xiong, et al., 1999 and Cai and Morishima, 2000).

Non-shattering is generally a recessive trait controlled by one or two genes in most cultivated species, for example one gene in rice and lentil, two genes in sorghum and barley, and wheat (Hancock, 1992). Oats are an exception. Spikelet non-shattering and floret non-disjunction of oat are controlled by one gene with dominant effect (Hancock, 1992). Four to five genes have been reported to affect seed shattering in rice using molecular analysis (Xiong, et al., 1999 and Cai and Morishima, 2000). Non-shattering in pear millet has been reported to be controlled by recessive alleles at three gene loci (Hancock, 1992).

In the genus *Fagopyrum*, the inheritance of seed shattering has been studied in the ancestor of cultivated common buckwheat *F. esculentum* ssp. *ancestrale* and in the wild species, *F. homotropicum*. Fesenko et al. (1998) and Ohnishi (1999) have reported that seed shattering in *F. esculentum* ssp. *ancestrale* was a dominant trait controlled by one gene, based on a segregation ratio of 3:1 (shattering: non-shattering) of F_2 progeny in the hybrid between non-shattering common buckwheat and *F. esculentum* ssp. *ancestrale*. In

the hybrid between non-shattering common buckwheat and shattering *F. homotropicum*, both Fesenko et al. (1998) and Wang and Campbell (1998) reported a ratio of 3:1 in the F_2 progeny, inferring that a single gene controls seed shattering in *F. homotropicum*. However, these reports did not separate F_1 plants individually for the study. If the segregation of the F_2 was derived from bulked F_1 hybrid plants, the results might not reflect the real genetic pattern, as *F. esculentum* is a heterogeneous population due to cross-pollination.

2.5.3 Inheritance of Winged Seed in Buckwheat

Fagopyrum species have triangular seeds that are either non-winged or winged. The winged character is an extension of the hull, usually at the widest portion of the seed. Winged seed is most common in wild species as it increases the range of seed dispersal. Winged seeds occur to a limited extent in the two cultivated species, common and Tartary buckwheat, and therefore, there have been very few studies on this character.

The inheritance of winged seed has become important in buckwheat breeding, as *F. homotropicum*, a wild species with mostly winged seed, was used to improve common buckwheat. Winged seed reduces seed density and causes problems with cleaning equipment. There have been two reports found on the inheritance of this character. In interspecific hybrid between non-winged *F. esculentum* and winged *F. homotropicum*, a ratio of 192 winged: 96 non-winged seeds in the F_2 segregation from the bulked F_1 had no clear genetic pattern (Wang and Campbell, 1998). In the same type of cross, Zeller and Hsam (2001) recorded a ratio of 9:7 in their F_2, indicating that two complementary gene might control winged seed. Therefore, it was necessary to clarify the inheritance of winged seed in buckwheat.

In short, studies in *Fagopyrum* have achieved great success in plant breeding, genetics, and phylogeny, but many questions remain to be answered. *Fagopyrum homotropicum* is playing a remarkable role in common buckwheat improvement due to its self-compatibility and the close relationship with common buckwheat. However, this species is complex with the existence of two ploidy forms. Is tetraploid *F. homotropicum*

autoploid or alloploid in origin? Both *F. homotropicum* and Tartary buckwheat are self-compatible. Is Tartary more easily crossed with *F. homotropicum* than with common buckwheat? Is it possible to use *F. homotropicum* as a bridge species to connect the two cultivated species in hybridization? *Fagopyrum homotropicum* diploid has been used in common buckwheat improvement through interspecific hybridization. What is the potential of *F. homotropicum* tetraploid in buckwheat improvement to increase genetic variability? Direct crossing of the two cultivated species has met with little success. Is it possible through different approaches to overcome the difficulty of crossing to improve the hybrid fertility? Self-incompatibility, seed shattering and winged seed are present in interspecific hybrids. The design of an effective breeding program to produce buckwheat with self-compatibility, non-shattering, and non-winged seed will depend on the understanding of these characteristics on a genetic basis. However, the current inheritance models are incomplete and no complete genetic studies have been conducted. The study of these characters will be critical for the success of buckwheat breeding through interspecific hybridization.

3.0 MANUSCRIPTS

MANUSCRIPT 1

Intraspecific and Interspecific Crossability and Cytological Characterization in Diploid and Tetraploid Forms of *Fagopyrum homotropicum*

ABSTRACT

Fagopyrum homotropicum is a species that is closely related to cultivated species *F. esculentum* but has the same breeding system as another cultivated species *F. tataricum*. It has diploid (2n = 2x = 16) and tetraploid (2n = 4x = 32) forms. Hybrids were developed from both intraspecific crosses within *F. homotropicum* and interspecific crosses between *F. homotropicum* and the two cultivated species. Accessions that had the same ploidy level within *F. homotropicum* were easily crossed but, with one exception, seed abortion occurred. *Fagopyrum homotropicum* produced hybrids with both *F. esculentum* and *F. tataricum* through ovule rescue. This indicates that *F. tataricum* might be closer to *F. homotropicum* than to *F. esculentum* because the direct cross of the two cultivated species is difficult. The hybrids between *F. esculentum* and *F. homotropicum* 2x were fertile, suggesting that the two species are very closely related. In the intraspecific and interspecific crosses, hybrids between the 2x and 4x forms of *F. homotropicum* were sterile with 24 chromosomes (eight chromosomes from the 2x form and 16 chromosomes from the 4x form). *Fagopyrum tataricum* was more compatible with *F. homotropicum* 2x than to *F. homotropicum* 4x, but *F. esculentum* was more compatible with *F. homotropicum* 4x than to *F. homotropicum* 2x. Both unilateral and bilateral compatibility exist between *F. homotropicum* and *F. esculentum*. The allotetraploid origin of *F. homotropicum* 2n = 4x = 32 was evident based on normal pollen viability and high seed productivity, stable chromosome number, normal chromosome pairing at meiosis, and the karyotype with two distinct groups of eight somatic chromosomes.

INTRODUCTION

Fagopyrum homotropicum is a wild species that was collected by Ohnishi (1995) in the Southwest of China. This species is self-compatible with homostyle flowers similar to 4one of the two cultivated species *F. tataricum* (2n = 2x = 16), and distinct from another cultivated species, *F. esculentum* (2n = 2x = 16) that is self-incompatible with the distylic flower types of pin and thrum. Based on phylogenetic study, all three species belong to the cymosum group in *Fagopyrum* genus, but *F. homotropicum* is closely related to *F. esculentum* (Ohnishi and Matsuoka, 1996). Therefore, the potential of *F. homotropicum* in buckwheat improvement through interspecific hybridization is of interest. It is assumed that *F. tataricum* might be easier to cross with *F. homotropicum* than with *F. esculentum* because both *F. tataricum* and *F. homotropicum* are self-compatible. If this is true, *F. homotropicum* can be used to improve the two cultivated buckwheat and as a bridge species to connect the two cultivated species because direct cross between the two cultivated species is difficult (Morris, 1951; Samimy, 1991; Samimy et al., 1996; and Wang and Campbell, 1998).

A total of 19 populations of *F. homotropicum* have been collected and studied using morphological, isozyme and cytological analysis (Ohnishi and Asano, 1999). Three populations are tetraploid (2n = 4x = 32) and the remainders are diploid (2n = 2x = 16). Allotetraploid origin of *F. homotropicum* 4x has been suggested by Ohnishi and Asano (1999) based on isozyme variability. One of progenitors might be *F. esculentum* spp. *ancestrale* that is the ancestor of cultivated buckwheat *F. esculentum* and another progenitor probably is a population of *F. homotropicum* 2x. The effort to confirm this hypothesis using *in situ* hybridization (Asano, 1998) failed due to indistinguishable chromosomes. Chromosome banding techniques are not applicable in *Fagopyrum* species because of the small size of chromosomes and the difficulty in chromosome preparation. Therefore, other approaches such as chromosome karyotype and chromosome pairing should provide basic and relevant evidence to confirm the hypothesis.

The objectives of this study were: 1) to hybridize *F. homotropicum* with the two cultivated species in order to use *F. homotropicum* as a bridge species to overcome the

difficulty of the direct cross; 2) to determine the relationships among *F. homotropicum*, *F. esculentum*, and *F. tataricum* based on crossability; 3) to compare the diploid with the tetraploid forms of *F. homotropicum* in order to utilize them in buckwheat breeding; 4) to provide evidence of the origin of *F. homotropicum* 4x based on pollen viability and cytological observations.

MATERIALS AND METHODS

Plant materials and types of crosses. Twelve accessions of the three species, *F. homotropicum* (six accessions), *F. esculentum* (five accessions), and *F. tataricum* (one accession) were used in the study. Seven types of crosses were made, including three intraspecific crosses within *F. homotropicum* (2x /2x, 4x / 4x, and 2x /4x) and four interspecific crosses (*F. esculentum* / *F. homotropicum* 2x and 4x, and *F. tataricum* / *F. homotropicum* 2x and 4x) (Table 3.1.1). Flowers of the female plants were emasculated one day before pollination or in the early morning prior to pollination. In crosses of 2x by 4x, ovules were harvested prior to abortion and were cultured *in vitro* approximately 10-14 days after pollination as previously described (Wang and Campbell, 1998). All plants were grown in a greenhouse maintained at a minimum of 22°C temperature with natural light supplemented to a 16/8 h day/night photoperiod using high-pressure sodium 400 Walt light.

Cytological analysis. Root tips were sampled from germinated seeds or directly from established plants, pretreated in a solution of 0.15 ml (3 drops) bromonapthalene in one ml water of for 1.5 h at room temperature, and then fixed in Farmer's fixative (3:1 95% ethanol: acetic acid) for at least one day. The root tips were hydrolyzed in 3N HCl at 60°C for 20 min., and stained by 2% acetocarmine, and then squashed in a drop of 45% acetic acid on a slide for observation under a light microscope. Young flower buds were collected at meiosis, stained by acetocarmine, squashed, and observed under the light microscope.

Pollen viability. Pollen grains were stained using an iodine solution containing 0.05% iodine and 0.1% potassium iodide. Approximately 500 to 900 pollen grains were checked

in each hybrid or parent plants. Pollen grains that were large and stained blue were considered to be viable, while small and non-stained pollen grains were scored as inviable.

Calculation of crossability, the percentage of hybrid recovery, and pollen stainability. Three methods were used to assess the relationship between the species in the hybrids. Crossability was determined after the cross was made.

Crossability (%) = (the number of enlarged ovaries / the total number of flowers pollinated) x100

If seed abortion occurred in the cross, ovules were cultured *in vitro*. The hybrid recovery was used to assess the percentage of hybrid plants obtained from the ovules:

Hybrid recovery (%) (HBR) = (the number of hybrids obtained / the total number of ovules rescued) x 100

Pollen stainability was used to assess the viability of the hybrids:

Pollen stainability (%) (PS) = (the number of pollen grains stained / the total number of pollen grains sampled) x 100

Chi-square analysis (Zar, 1999) was used to compare significance of the two types of crosses in crossability and hybrid recovery as the following:

$\chi^2 = (f_{11}f_{22} - f_{12}f_{21} \pm n/2)^2 \times n/(R_1.R_2.T_1.T_2)$

For example, to compare crossability in two types of crosses:

f_{11} and f_{12}: the umber of flowers fertilized (f_{11}) and non-fertilized (f_{12}) in cross type 1;

f_{21} and f_{22}: the number of flowers fertilized (f_{21}) and non-fertilized (f_{22}) in cross type 2;

T_1 and T_2 = the total number of flowers pollinated in cross type 1 (T_1) and in cross type 2 (T_2);

R_1 and R_2 = the total number flowers fertilized (R_1) and non-fertilized (R_2) in the two types of crosses;

n = the total number of flowers pollinated in two types of crosses.

RESULTS

In total, 219 hybrids were produced from 32 intraspecific and interspecific crosses in the seven types of cross combinations (Table 3.1.1). The crossability, the percentage of hybrids recovered from rescued ovules, and pollen stainability in the crosses are reported in Table 3.1.2 and 3.1.3.

Table 3.1.1. Hybrid plants produced from intraspecific crosses within *F. homotropicum* and interspecific crosses among *F. homotropicum* (H), *F. esculentum* (E) and *F. tataricum* (T).

Cross	# of hybrids	Cross	# of hybrids
1. *F. homotropicum* 2x / 2x		K950818-1 (H) / BM940364 (E)	4
K950818-1 / K950818-3	2	K980854 (H) / BM940364 (E)	21
K980855 / K980854	59	X980088-7p (E) / K980854 (H)	2
K980854 / K980855	27	K980855 (H) / BM940364 (E)	9
K980855 / K950818-1	1		
K950818-1 / K980855	1	5. *F. esculentum* / *F. homotropicum* 4x	
K950818-3 w / K950818-3nw	17	K950818-2 (H) / 509 (E)	2
K950818-3 nw / K950818-3 w	13	509 (E) / K950818-2 (H)	16
		BM940172 (E) / K950818-2 (H)	14
2. *F. homotropicum* 4x / 4x		BM930284 (E) / K950818-2 (H)	5
K950818-2 / K950818-4	4	BM940364 (E) / K950818-4 (H)	4
		K950818-4 (H) / BM940364 (E)	1
3. *F. homotropicum* 2x / 4x			
K950818-2 (4x) / K950818-3 (2x)	1		
K950818-1 (2x) / K950818-4 (4x)	2	6. *F. tataricum* / *F. homotropicum* 2x	
K950818-1 (2x) / K950818-2 (4x)	1	B770198(T) // K950818-1(H) / K950818-3(H)	3
K950818-2 (4x) / K950818-1 (2x)	0	B770198 (T) / K950818-1 (H)	2
K950818-3 (2x) / K950818-4 (4x)	1	B770198 (T) / K950818-3 (H)	0
K950818-3 (2x) / K950818-2 (4x)	0		
K950818-4 (4x) / K950818-3 (2x)	5	7. *F. tataricum* / *F. homotropicum* 4x	
		B770198 (T) / K950818-2 (H2)	1
4. *F. esculentum* / *F. homotropicum* 2x		B770198 (T) / K950818-4 (H2)	1
BM940364 (E) / K950818-1 (H)	0	K950818-2 (H2) / B770198 (T)	0
Total			219

Table 3.1.2. Crossability, the percentage of hybrid recovery and pollen stainability in intraspecific and interspecific crosses of buckwheat species and pollen stainability in the two forms of *F. homotropicum* diploid 2x and tetraploid 4x.

Cross	number of flowers pollinated	number of ovules rescued	number of hybrids	Crossability %	HBR %	PS %
F. h 2x / *F. h* 4x	245	33	10	13.5	30.3	17.5
F. e / *F. h* 2x	1115	145	36	13.0	24.8	81.4
F. e / *F. h* 4x	278	94	-	33.8	-	-
		194	41	-	21.1	22.3.
F. t / *F. h* 2x	79	28	5	35.4	17.9	2.9
F. t / *F. h* 4x	41	7	-	17.1	-	-
	-	11	2	-	18.2	-
F. homotropicum 2x						99.6
F. homotropicum 4x						99.5

F. h: *F. homotropicum*; *F. e*: *F. esculentum*; *F. t*: *F. tataricum*. HBR: hybrid recovery; PS: pollen stainability. Missing values (-) indicate no data recorded.

Intraspecific hybridization within F. homotropicum. Three types of crosses were made within *F. homotropicum* including 2x / 2x, 4x / 4x, and 2x / 4x (Table 3.1.1). In most crosses, it was not difficult to cross and obtain fertile hybrids between accessions at the same ploidy level. One exception was the cross within 2x accessions between K980855 and K950818-1 in which most of the crossed seeds aborted, indicating that these two accessions were not very closely related within the same species. The 2x accessions were cross compatible with the 4x accessions, but the seeds did not grow to maturity and the ovules had to be rescued *in vitro*. The crossability and the percentage of hybrid recovery were different between the reciprocal crosses. For example, in the crosses between K950818-3 (2x) and K950818-4 (4x), the cross using K950818-4 as the female parent had a higher crossability and the percentage of hybrid recovery (Table 3.1.3). However, K950818-1 (2x) and K950818-2 (4x) were only cross compatible when K950818-1 was used as the female parent (Table 3.1.3).

Table 3.1. 3. Crossability and the percentage of hybrid recovered (HBR) in reciprocal and bridge crosses from *F. homotropicum* (H), *F. esculentum*, and *F. tataricum* (T).

Cross	Number of flowers pollinated	Number of ovules rescued	Number hybrids	Crossability %	HBR %
F. homotropicum 2x / 4x:	245	33	10		
K950818-1 (2x) / K950818-2 (4x)	71	7	1	9.9	14.3
K950818-2 (4x) / K950818-1 (2x)	31	0	-	0	-
K950818-3 (2x) / K950818-4 (4x)	39	3	1	7.7	33.3
K950818-4 (4x) / K950818-3 (2x)	38	8	5	21.1	62.5
F. esculentum / *F. homotropicum* (2x): unilateral incompatibility (SI/SC)					
BM940364 (SI) / K950818-1 (SC)	128	0	-	0	-
K950818-1 (SC) / BM940364 (SI)	67	10	4	14.9	40.0
F. esculentum / *F. homotropicum* (4x): bilateral compatibility					
BM940364 (SI) / K950818-4 (SC)	28	2	-	7.1	-
K950818-4 (SC) / BM940364 (SI)	47	2	-	4.3	-
509 (SI) / K950818-2 (SC)		71	16	-	22.5
K950818-2 (SC) / 509 (SI)		7	2	-	28.6
F. tataricum / *F. homotropicum* (2x):					
B770198 (T) / K950818-3 (H)	245	50	0	20.4	0
B770198 (T) / K950818-1 (H)	13	8	2	61.5	25.0
B770198 (T) // K950818-1 (H)/K950818-3 (H)	66	21	3	31.8	14.3

SC: self-compatibility; SI: self-incompatibility.

Interspecific hybridization between F. homotropicum and F. esculentum. Both 2x and 4x accessions of *F. homotropicum* were found to be cross-compatible with *F. esculentum* (Table 3.1.2). In *F. homotropicum* 2x / *F. esculentum*, all crosses were made in the direction of self-compatibility (SC) by self-incompatibility (SI) except for one cross between BM940364 (SI) and K950818-1 (SC) that was made in the both directions, SC / SI and SI / SC. This cross was cross compatible only in the direction SC / SI when the self-compatible parent, K950818-1, was used as the female parent crossed with self-incompatible parent BM940364. Ten seeds were produced from 67 pollinated flowers in the cross of SC / SI, but no seed was obtained from 128 pollinated flowers in the reciprocal cross of SI / SC (Table 3.1.3). This unilateral incompatibility often prevents self-incompatible species from accepting the pollen of species which reproduce through selfing (Nettancourt, 1977). However, in *F. esculentum* / *F. homotropicum* 4x, crosses were bilaterally crossable in both SC / SI and SI / SC (Table 3.1.3).

Interspecific hybridization between F. homotropicum and F. tataricum. Both 2x and 4x accessions of *F. homotropicum* were crossable with *F. tataricum*, but the 2x accessions had a higher crossability rate with *F. tataricum* (35.4%) compared to 4x (17.1%) (Table 3.1.2). It was observed that one 2x accession of *F. homotropicum*, K950818-3 was easily crossed with *F. tataricum*, but no hybrids were produced from the 50 ovules rescued (Table 3.1.3). However, another accession of *F. homotropicum* 2x, K950818-1, was not only crossable with *F. tataricum*, but also produced hybrids after ovule rescue. Eight seeds were produced from 13 pollinated flowers and two hybrids were produced using ovule culture (Table 3.1.3). Using K950818-1 as a bridge parent, three hybrids between K950818-3 and *F. tataricum* were produced through a cross with *F. tataricum* // K950181-1 / K950181-3 (Table 3.1.3).

Overall crossability and the percentage of hybrid recovery. In the 2x / 4x crosses, the percentage of hybrid recovery in intraspecific hybridizations within *F. homotropicum* was slightly higher at 30.3% than the rest of the interspecific crosses, with hybridization ranging from 17.9% to 24.8% (Table 3.1.2). *Fagopyrum tataricum* was more cross-compatible with the *F. homotropicum* 2x accessions (crossability 35.4%) than with the *F.*

homotropicum 4x accession (crossability 17.1%) (Table 3.1.2). In the contrast, *F. esculentum* was more compatible with the *F. homotropicum* 4x accession (crossability 33.8%) than that with the 2x accession of *F. homotropicum* (crossability 13.0%) (Table 3.1.2). Based on Chi-square analysis, there was no significant difference in hybrid recovery in all types of crosses (Table 3.1.4). However, in crossability, the significant difference between crosses of *F. esculentum/F. homotropicum* and crosses of *F. tataricum/F. homotropicum* reflected the difference between the two cultivated species (Table 3.1.4). Crossability in crosses of *F. esculentum* or *F. tataricum /F. homotropicum* 2x significantly differed from the crosses of *F. esculentum* or *F. tataricum / F. homotropicum* 4x (Table 3.4.1), indicating that the two forms of *F. homotropicum* had distinct responses.

Hybrid fertility and pollen viability expressed by pollen stainability. Intraspecific hybrids of *F. homotropicum* that were produced within the 2x or 4x accessions were fertile, while intraspecific hybrids between 2x and 4x accessions were sterile. Interspecific hybrids between *F. esculentum* and *F. homotropicum* 2x were fully fertile, but the hybrids from the rest of the interspecific crosses, including *F. esculentum / F. homotropicum* 4x and *F. tataricum / F. homotropicum* 2x or 4x, were sterile

The highest level of pollen stainability (81.4%) occurred in the fertile hybrid between *F. esculentum / F. homotropicum* 2x, and the lowest level (2.9%) was found in the hybrids between *F. tataricum / F. homotropicum* 2x (Table 3.1.2). The rest of the hybrids had similar levels of pollen stainability with an average of approximately 20% (Table 3.1.2). There was no difference in pollen stainability between *F. homotropicum* 2x (accession K980854) and 4x (accessions K950818-2 and K950818-4) at 99.6% and 99.5%, respectively (Table 3.1.2). Both types had normal seed set without seed abortion.

Cytology. In the crosses of 2x / 2x accessions, the F_1 hybrids had 16 chromosomes. In the crosses of 4x / 4x accessions, the progeny had 32 chromosomes. The F_1 hybrids from the crosses of 2x / 4x all had 24 chromosomes, eight chromosomes from the 2x parent and 16 chromosomes from the 4x parent.

Fagopyrum homotropicum 4x consistently had 32 somatic chromosomes and 16 bivalents at meiosis. Based on the position of centromeres, size and shape of chromosomes, and relative length of arms (Sinkovic and Bohanec, 1988; Schulz-Schaeffer, 1985; and Haskell, 1968), 32 chromosomes could be sorted into 16 pairs (accession K950818-2, Figure 3.1.1) The karyotype shows that seven chromosomes (1, 2, 3, 7, 11, 13, 15) were submetacentric and chromosome 16 was the shortest (Figure 3.1.1). The karyotype of the eight chromosomes of *F. homotropicum* 2x (accession K950818-1) corresponded to the karyotype of eight of the16 chromosomes of *F. homotropicum* 4x (accession K950818-2) (Figure 3.1.1 and 3.1.2 and Table 3.1.5) (size scale not available).

The morphology of the eight chromosomes from one of the accessions (509) of *F. esculentum* agreed with the *F. esculentum* karyotype described by Sinkovic and Bohanec (1988) with the exception that chromosome 4 was not obviously submetacentric as described. Six of the eight chromosomes from *F. esculentum* in this study corresponded well with six of the 16 chromosomes from *F. homotropicum* 4x, and the remaining two chromosomes 4 and 8 in *F. esculentum*, were similar to chromosome 9 and 15 in *F. homotropicum* 4x (Figures 3.1.1 and 3.1.3 and Table 3.1.5).

Table 3.1.4. Comparison of each two types of crosses in crossability and hybrid recovery.

Cross type	Crossability (number of)				Hybrid recovery (number of)			
	pollinated	enlarged	died	χ^2	rescued	survived	died	χ^2
F.t x *F.h* (2x)	79	50	29	4.06*	29	5	24	0.29
F.t x F.*h* (4x)	41	34	7		11	2	9	
F.e x *F.h* (2x)	1115	970	145	69.26**	145	36	109	0.45
F.e x *F.h* (4x)	278	184	94		194	41	153	
F.t x *F.h* (2x)	79		29	31.42**	29	5	24	1.25
F.e x*F. h* (2x)	1115		145		145	36	109	
F.t x *F.h* (4x)	41	34	7	5.43*	11	2	9	0.37
F.e x *F.h* (4x)	278	184	94		194	41	153	

*χ^2 (df =1, P = 0.05) = 3.84; **χ^2 (df =1, P = 0.01) = 6.63

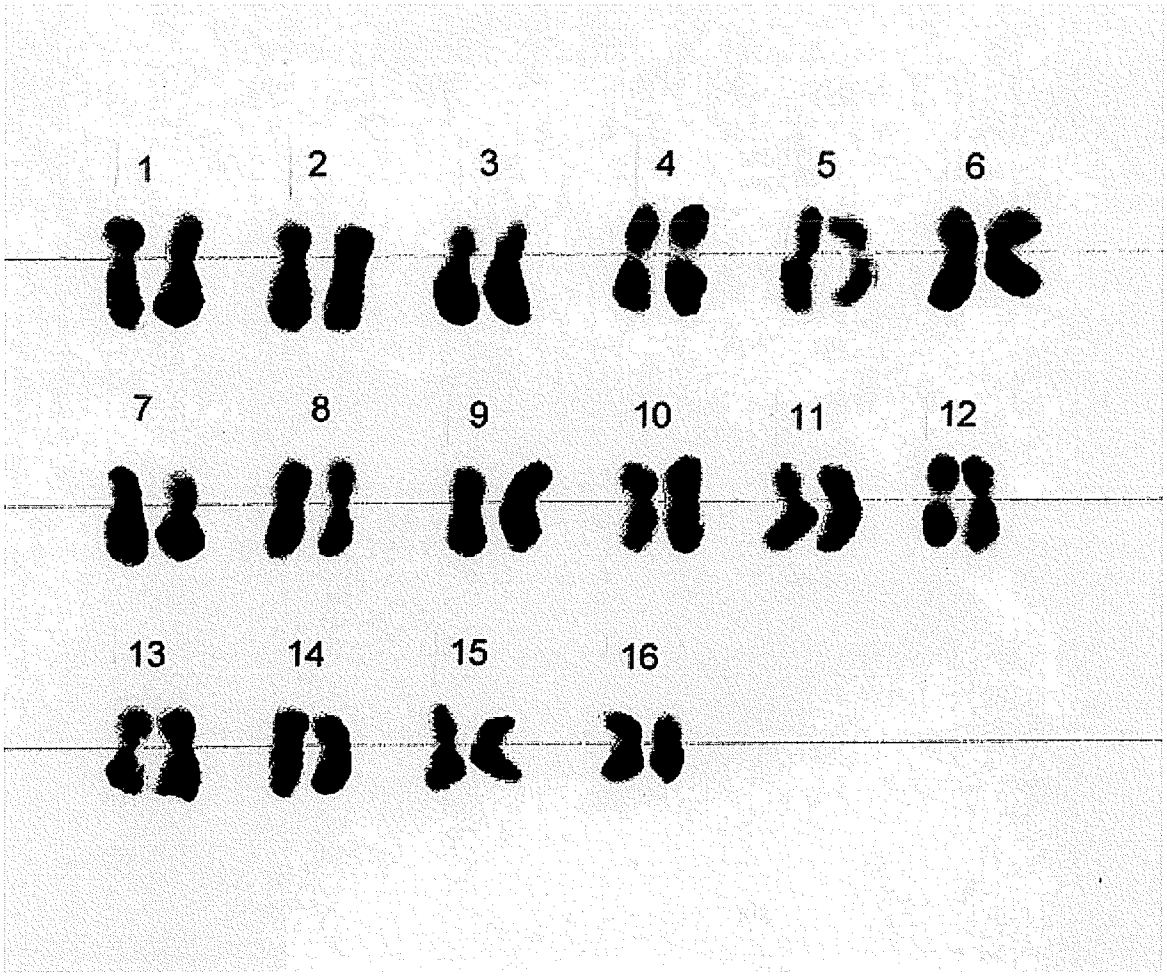

Figure 3.1.1. The karyotype of *F. homotropicum* tetraploid, accession K950818-2, with 16 pairs of chromosomes.

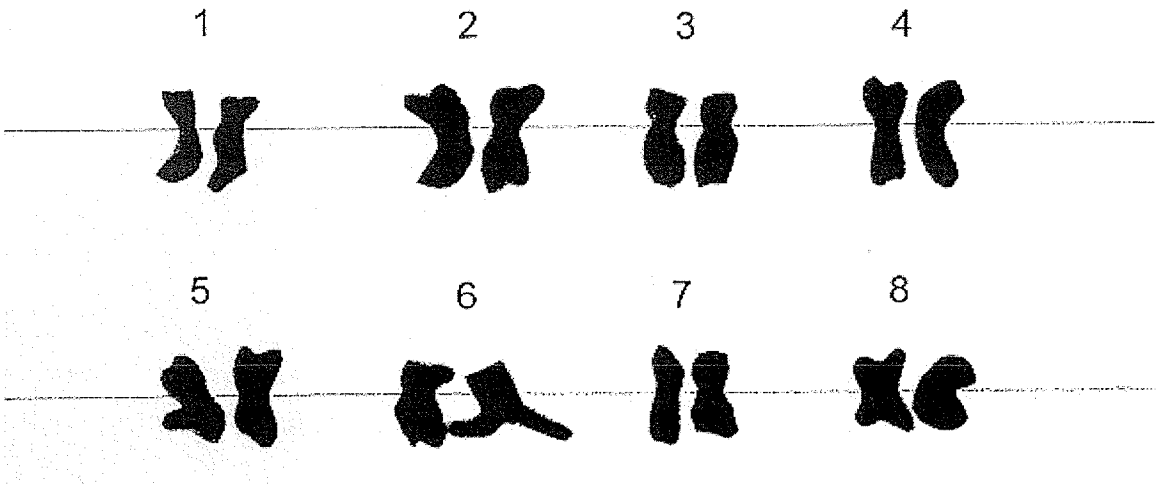

Figure 3.1.2. The karyotype of *F. homotropicum* diploid, accession K950818-1, with eight pairs of chromosomes.

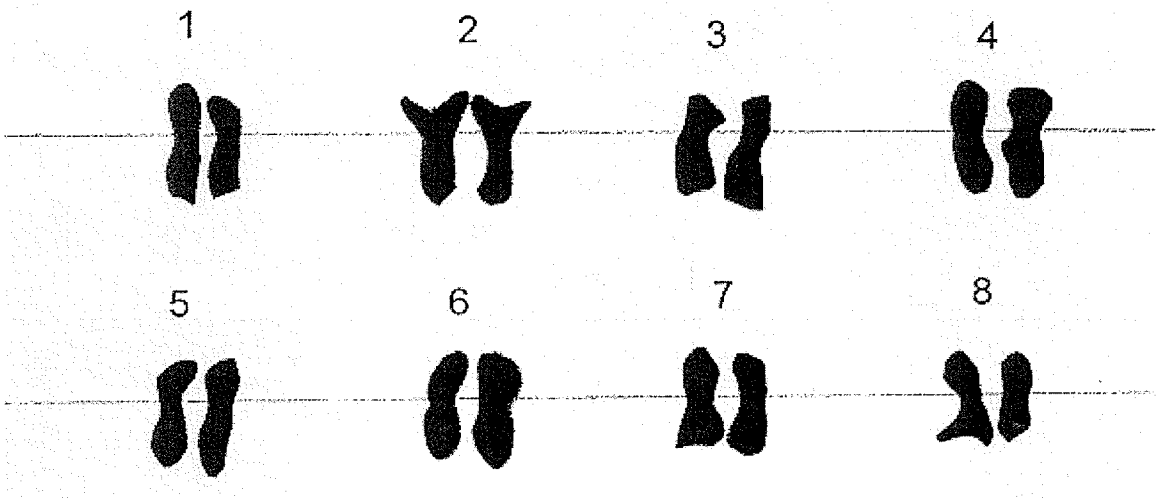

Figure 3.1.3. The karyotype of *F. esculentum*, accession 509, with eight pairs of chromosomes.

Table 3.1.5. The correspondence between the 16 chromosomes of *F. homotropicum* 4x and the eight chromosome complement of *F. homotropicum* 2x and *F. esculentum*.

Chromosome No. (*F. homotropicum* 4x)	Chromosome No. (*F. homotropicum* 2x)	Chromosome No. (*F. esculentum*)
1 sb		2
2 sb	3	
3 sb		3
4	4	
5		1
6	2	
7 sb		5
8	7	
9		4?
10	5	
11 sb	1	
12		6
13 sb	6	
14		7
15 sb		8?
16	8	

sb: submetacentric.

DISCUSSION

The relationships between accessions and species based on hybridization. This study provides information on the phylogenic relationships in *Fagopyrum* between species and between accessions within species. Within *F. homotropicum*, the low crossability between the two accessions at the same ploidy level indicated that two accessions might not be closely related within the species, for example, K950818-1 and K980855. The fertile hybrid between *F. esculentum* and *F. homotropicum* 2x indicated that these two species might be very closely related. *Fagopyrum homotropicum* 2x and 4x was crossable

with *F. tataricum*, while *F. esculentum* is difficult to cross with *F. tataricum* (Samimy et al., 1996 and Wang and Campbell, 1998), suggesting that *F. tataricum* might be more closely related to *F. homotropicum* than to *F. esculentum*. This supports the possibility of using *F. homotropicum* as a bridge species to hybridize the two cultivated species. The sterility of the 2x / 4x hybrids in both intraspecific and interspecific crosses was due to triploidy resulting in the failure of chromosome pairing. In comparison of the crossability in interspecific crosses, *F. homotropicum* 2x was more compatible with *F. tataricum*, but *F. homotropicum* 4x was more compatible with *F. esculentum* (Table 3.1.2). *Fagopyrum homotropicum* 2x and *F. esculentum* have the same ploidy level and share a similar genetic background as shown by the production of the fertile hybrids. However, it is not clear why *F. homotropicum* 2x had a lower crossability than *F. homotropicum* 4x when crossed with *F. esculentum* (Table 3.1.2).

Unilateral incompatibility and bilateral compatibility. Unilateral incompatibility (SI x SC) has been widely documented in plants (Nettancourt, 1977) when a self-compatible species was crossed with a self-incompatible species. In this study, one cross between *F. esculentum* and *F. homotropicum* 2x showed unilateral incompatibility (Table 3.1.3). However, bilateral compatibility has been reported by Fesenko, I.N., et al. (2001) in the same type of crosses. This may be genotype dependent. It was apparent that the crosses between *F. esculentum* and *F. homotropicum* 4x were bilaterally compatible (Table 3.1.3).

Evidence of allotetraploid origin of F. homotropicum 4x. Ohnishi and Asano (1999) have suggested an allotetraploid origin for *F. homotropicum* 4x based on isozyme analysis due to fixed heterozygosity and bigenic disomic inheritance. This study provides additional evidence in support of this hypothesis.

Autotetraploids often express 'gigas' morphological characters with larger plants and darker green leaves, in comparison with the original 2x species (Singh, 1993). Even though the *F. homotropicum* 4x are taller than that the 2x, there are no obvious differences in leaf size or color. Autotetraploids are rarely used as seed producers

because of their lower fertility compared with the 2x form, resulting from meiotic disruption (Simmonds, 1979). The seed productivity of *F. homotropicum* 4x is the same as 2x form and pollen stainability of the 4x pollen (99.5%) was as high as the 2x (99.6%) (Table 3.1.2).

The chromosome number of *F. homotropicum* 4x was consistent and no aberrant chromosomes were observed in both this study and Ohinish and Asano's study (1999). Autotetraploids often have variable chromosome numbers as a result of multivalent formation causing abnormal segregation at meiosis. In both intraspecific and interspecific crosses of 2x / 4x, all of the F_1 hybrids had 24 chromosomes, indicating that the 16 chromosomes derived from *F. homotropicum* 4x were stable with the formation of normal gametes n = 16. The occurrence of 16 bivalents at meiosis supports the proposed allotetraploid origin, as autotetraploids often have higher levels of pairing producing trivalents and quadrivalents. If *F. homotropicum* 4x were autotetraploid in origin, the 32 chromosomes would be made up of eight groups with four homologous chromosomes in each group. However, the 32 chromosomes from the accession of *F. homotropicum* 4x in our study were grouped into 16 pairs of homologous chromosomes, confirming the allotetraploid origin. All evidence supports the origin of *F. homotropicum* 2n = 4x = 32 as an allotetraploid rather than an autotetraploid.

Proposed progenitors of F. homotropicum tetraploid. Based on isozyme analysis, Ohnishi and Asano (1999) suggested that two possible progenitors of *F. homotropicum* 4x could be a 2x population of *F. homotropicum* and *F. esculentum* ssp. *ancestrale* that is the ancestor of cultivated *F. esculentum*. In this study, the comparison of the karyotype of *F. homotropicum* 2x and *F. esculentum* with the karyotype of *F. homotropicum* 4x, provided support for this hypothesis. Two chromosomes of *F. esculentum* showed similarity to the two chromosomes of *F. homotropicum* 4x, with a possible difference caused by evolutionary changes between *F. esculentum* and its ancestor *F. esculentum* ssp. *ancestrale*. Therefore, further comparisons should be made between the karyotype of *F. homotropicum* 4x with *F. esculentum* ssp. *ancestrale*. It will be necessary to hybridize between *F. homotropicum* 2x and *F. esculentum* ssp. *ancestrale* directly to

definitively prove the hypothesis. Since precise cytological techniques like chromosome banding is not available and failed in use advanced cytological technique such as *in situ* hybridization (Asano, 1998) in *Fagopyrum*, molecular technique, for example microsatellite, might be another option to confirm this hypothesis.

Classification of F. homotropicum. In taxonomic classification, species are recognized by obvious characters which serve as key features for the purpose of identification. As a bridge species between two cultivated species, *F. homotropicum* is distinguished from *F. tataricum* by cotyledon characteristics and from *F. esculentum* by floral morphology (Ohnishi, 1995). There are no obvious characters to differentiate between the 2x and 4x forms of *F. homotropicum* even though these two types have different genetic backgrounds and are at the different ploidy levels. Therefore, this study suggests that 4x should remain classified as *F. homotropicum* as is the case of *Glycine tomentella* that has both diploid (2n = DD = 40) and allotetraploid forms (2n = AADD = 80) (Singh, 1993). In this way, the 4x could be different from 2x at the infraspecific level. In terms of phylogeny, this implies that *F. homotropicum* should be classified as a polyphyletic species rather than a monophyletic species. Another alternative is to consider the 2x and 4x types as microspecies or sibling species that have minor differences in morphology but are reproductively isolated (Judd, et al., 1999).

MANUSCRIPT 2

Cytological Characterization of Interspecific Hybrids between *Fagopyrum esculentum* (2n = 2x = 16) and *F. homotropicum* (2n = 4x = 32)

ABSTRACT

Interspecific hybrids between the diploid species *Fagopyrum esculentum* (2n = 2x = 16) and the tetraploid species *F. homotropicum* (2n = 4x = 32) were obtained using ovule rescue. The F_1 hybrids were found to be triploid (2n = 3x = 24) with a high level of sterility. However, two of 41 plants set one seed each. After the plants were grown for approximately two years maintained by cuttings, one of these two plants set another five seeds and a third plant set one seed. Fertility was also restored using colchicine treatment, which normally results in chromosome doubling. The F_2 progeny were determined to be hexaploid (2n = 6x = 48), diploid (2n = 2x = 16), and aneuploid (2n = less or more than 48). The F_3 progeny of the hexaploid plants had variable chromosome numbers from 46 to 50, presumed to be the result of unbalanced gamete formation. In the F_2 diploid plants, eight chromosomes of the F_1 hybrids were eliminated. These diploid plants showed morphological characters from both *F. esculentum* and *F. homotropicum*, suggesting that the plants might contain chromosomes or chromosome fragments from both parents. In addition, a plant with 17 chromosomes that could be a trisomic or an addition line was found in this study. The potential applications of these lines for buckwheat improvement and future genetic studies are discussed.

INTRODUCTION

Fagopyrum homotropicum is a self-compatible wild species that was collected from the Southwest China by Ohnishi (1995). Both diploid (2n = 2x = 16) and tetraploid (2n = 4x = 32) forms have been found in this species (Asano, 1998). Campbell (1995), Wang and Campbell (1998) and Woo et al. (1999a) have reported successful interspecific hybridization between *F. esculentum* (2n = 2x = 16) and *F. homotropicum* diploid. These crosses were used to transfer valuable characteristics from *F. homotropicum* to *F. esculentum*, including frost tolerance, higher rutin content and increased expression of

green testa. At the same time, the self-compatibility character was introgressed from *F. homotropicum* into *F. esculentum*. Sterility has limited the utilization of the new hybrids developed between *F. esculentum* and *F. homotropicum* tetraploid (Wang and Campbell, 1998) in buckwheat breeding. Several seeds were harvested directly from the hybrid plants and later from the plant maintained by cuttings. The objectives of this study were to restore hybrid fertility through colchicine treatment and further to observe cytological characters of the progeny in order to utilize hybrids in buckwheat improvement.

MATERIALS AND METHODS

Hybrid development. Four accessions of *F. esculentum* (BM940364, BM940172, BM930284, and 509) and two accessions of *F. homotropicum* (K950818-2 and K950818-4) were used for crossing. The pin plant of *F. esculentum* was used as the female. Hybrids from four interspecific crosses (BM940364 / K950818-4, BM940172 / K950818-2, BM930284 / K950818-2, and 509 / K950818-2) were obtained by ovule rescue as described by Wang and Campbell (1998). The F_1 hybrids were grown in a greenhouse maintained at a minimum temperature of 22°C with natural light supplemented to a 16 h / 8 h day/light photoperiod. Sterile plants were maintained by cutting short stem sections with 2-3 leaves, dipping the cut end into 0.4 % rooting powder IBA (Plant Products Co. LTD. Rampton Ontario Canada L6T 1G1) and then planting the cuttings in covered growing containers with fine vermiculate under high humidity until roots formed.

Chromosome doubling. Seven of the 41 hybrids were picked from four crosses for chromosome doubling. Cotton balls were soaked in an aqueous solution of colchicine 0.1% (w/v) and were then placed on the axillary buds of the juvenile plants obtained from stem cuttings. The treatment was maintained for 24 h in a mist chamber. The misting system was set for 6 seconds at 32 min. intervals for the first hour and then for 4 seconds at 64 min. intervals for the remained 23 h.

Determination of chromosome number. Root tips were collected from germinated seeds and plant roots. Seeds were germinated in a Petri dish on moistened paper towels and root

tips were collected 3-4 days after germination. Root tips were collected from cuttings approximately 7-10 days after root formation (as described above).

Root tips were pretreated in a solution of 0.15ml/ml water of bromonapthalene for 1.5 h at room temperature and then were fixed in Farmer's fixative (95% ethanol: acetic acid = 3:1) for at least one day. The root tips were then hydrolyzed in 3N HCl at 60°C for 20 min., and stained by 2% acetocarmine, and then squashed in a drop of 45% acetic acid on a slide for observation under a light microscope (x1250).

The chromosome number of the F_1 hybrids was determined from the root tips of plant cuttings. Seeds from plants that had spontaneously doubled or had been treated with colchicine were used for chromosome determination in the F_2 generation. The root tips of 31 F_2 seeds were randomly selected for chromosome counting (Table 3.2.1).

To confirm the stability of the observed ploidy levels, the chromosome number was determined for 11 F_3 plants derived from a hexaploid F_2 plant with 48 chromosomes and five F_3 plants derived from a diploid F_2 plant with 16 chromosomes (Table 3.2.1). The chromosome numbers of the F_3 and F_4 generation from the original plant with 17 chromosomes were also determined (Table 3.2.1).

RESULTS

Hybrid fertility. The 41 F_1 hybrid plants obtained from the four interspecific crosses had self-compatible homostyle flowers resembling the male parent, *F. homotropicum.* All of the F_1 plants were sterile except for two plants that set one seed each. After approximately two years' maintenance using stem cuttings, one of the two plants set another five seeds and the third plant set one seed (Table 3.2.1).

Table 3.2.1. The chromosome numbers of the F_2, F_3, and F_4 generation in four interspecific crosses between *F. esculentum* (2n = 2x = 16) and *F. homotropicum* (2n = 4x = 32)

	Parents		F2		F3		F4	
Treatment	*F.esculentum*	*F.homotropicum*	# of plants	# of chr.	# of plants	# of chr	# of plants	# of chr
Non-colch.	BM940172	K950818-2	1	48				
			1	44				
			5	16				
Colch.	BM930284	K950818-2	5	48				
	BM940172	K950818-2	4	48				
			1	45				
	509	K950818-2	8	48	4	48		
					1	46		
					2	47		
					2	49		
					2	50		
	BM940364	K950818-4	1	17	8	17	4	17
					19	16	17	16
			1	16	5	16		
			3	46				
			1	48				
Total			31				21	

Colch.: colchicine; chr.: chromos

Seven of 41 sterile F_1 hybrids were restored to fertility by colchicine treatment. Seed set was variable, from less to more than 10 seeds formed per branch arising from the treated axillary bud.

Determination of chromosome number. As expected, the F_1 hybrid plants had 24 chromosomes, indicating that they were triploid (2n = 3x = 24), with one set of chromosomes from diploid *F. esculentum* (2n = 2x = 16) and two sets of chromosomes from tetraploid *F. homotropicum* (2n = 4x = 32).

The F_2 produced from the non-treated and treated F_1 plants were at three ploidy levels: hexaploid, diploid and aneuploid (Table 3.2.1). The majority, 19 out of 31 plants, was hexaploid (2n = 6x = 48) resulting from doubling the chromosome numbers of the F_1 hybrids. Six plants were diploid (2n = 2x = 16) (Figure 3.2.1A) with the same chromosome number as the *F. esculentum* parent. The remaining 6 plants were aneuploid. Five plants had 44 to 46 chromosomes and one plant had 17 chromosomes (Figure 3.2.1D).

To test stability of the chromosome complement, the progeny from the F_2 of hexaploid (2n = 6x = 48), diploid (2n = 2x = 16), and aneuploid (2n = 2x +1 = 17) plants were examined. Eleven F_3 plants derived from a hexaploid F_2 plant had chromosome numbers varying from 46 to 50 (Table 3.2.1 and Figure 3.2.1B and 1C). This indicated that unbalanced gametes had been produced, due to multivalent and univalents formation during meiosis. Diploid chromosome numbers were maintained in the F_3 plants from the F_2 diploid (2n = 2x = 16) (Table 3.2.1), indicating that regular bivalents had formed during meiosis. The F_3 and F_4 progeny derived from the plant with 17 chromosomes had either 16 or 17 chromosomes (Table 3.2.1), presumably resulting from n and n+1 gametes at meiosis.

Figure 3.2.1. Chromosome numbers of the interspecific hybrids between *F. esculentum* (2n = 2x = 16) and *F. homotropicum* (2n = 4x = 32) in mitotic metaphase. A: 16 chromosomes; B: 50 chromosomes; C: 45 chromosomes; D: 17 chromosomes.

Morphological characters of the plants with different chromosome numbers. The F_2 and F_3 diploid plants were fully fertile if the flowers were homostyle, the same form as the self-compatible parent, *F. homotropicum.* Morphological characters from both parents were clearly observed in these plants. For example, the plants had homostyle flowers resembling *F. homotropicum*, but the seeds did not shatter, a character of *F. esculentum.* The F_2 and F_3 hexaploid or aneuploid plants were partially fertile, and taller and lusher with larger seeds, thicker leaves and darker leaf color, compared with their diploid parents. These characteristics were similar to the so-called the 'gigas' feature that was observed in autopolyploids of other species (Simmonds, 1979). The F_3 and F_4 plants with 17 chromosomes ($2n = 2x + 1 = 17$) had low fertility and grew slowly, compared with their parents and the diploid plants.

DISCUSSION

Ploidy levels. In the triploid F_1 hybrid ($2n = 3x = 24$), one set of chromosomes was presumed to be derived from the diploid parent *F esculentum* ($2n = 2x = 16$) and two sets of chromosomes from the tetraploid parent *F. homotropicum* ($2n = 4x = 32$). The origin of the tetraploid *F. homotropicum* has not yet been confirmed as either auto- or allopolyploidy. Based on isozyme analyses, Asano (1998) reported that the *F. homotropicum* with 32 chromosomes probably is an allotetraploid, resulting from hybridization between a population of diploid *F. homotropicum* and *F. esculentum* spp. *ancestrale,* the ancestor of *F. esculentum.* In this case, one of two genomes in tetraploid *F. homotropicum* should resemble the genome of *F. esculentum.* Using A to represent the genome of *F. esculentum*, and A' and B to represent the two genomes of *F. homotropicum*, the genotype of the F_1 hybrid would be AA'B a segmental allopolyploid (Figure 3.2.2). After chromosome doubling, the ploidy level of the plants with 48 chromosomes would be represented as AAA'A'BB, an autoallohexaploid. Therefore, they would be expected to show the 'gigas' feature of an autopolyploid due to unbalanced chromosome division during meiosis resulting in aneuploid progeny.

Chromosome elimination. In this study, chromosome elimination occurred during the formation of gametes in the F_1 hybrid, resulting in F_2 progeny with chromosome numbers

reduced from 24 (2n = 3 x = 24) to 16 (2n = 2x = 16). Chromosome elimination has been documented in other interspecific or intergeneric crosses, i.e., barley x *Hordeum bulbosum*, wheat x *H. bulbosum*, wheat x maize, wheat x pearl millet and oat x maize crosses (Singh, 1993). However, in these cases, chromosome elimination occurs as the F_1 hybrid formed rather than after the F_1 hybrid developed.

The loss of eight chromosomes in the reduction from 24 to 16 is equivalent to the elimination of one complete genome. However, recombinant morphological characters were observed in the progeny. There are two possible chromosome complements in the F_2 progeny (Figure 3.2.2). One of the two genomes from *F. homotropicum* could have been eliminated, resulting in the genotype AA' or AB. Alternatively, the chromosomes of *F. esculentum* could be eliminated after recombination and the remaining two genomes of *F. homotropicum* may carry the fragments of *F. esculentum* chromosomes resulting in the genotype of A'_AB_A. In both cases, the phenotypes of the diploid F_2 plants would have recombinant morphological characters.

The occurrence of fertile plants after chromosome elimination in this study may be explained by the close relationship between *F. esculentum* and *F. homotropicum,* based on isozyme variability (Ohnishi and Matsuoka, 1996) and the nucleotide sequences of the rbcL and accD genes and their intergenic region (Yasui and Ohnishi, 1998). The production of fertile hybrids between *F. esculentum* and *F. homotropicum* diploid (Campbell, 1995 and Wang and Campbell, 1998) also indicated that the two species were closely related. Therefore, regardless of the way in which the chromosome elimination occurred in this study or whether tetraploid *F. homotropicum* (2n = 4x = 32) is an auto- or allotetraploid, the chromosomes of the diploid hybrid progeny would be expected to exhibit bivalent pairing, leading to production of the fertile progeny.

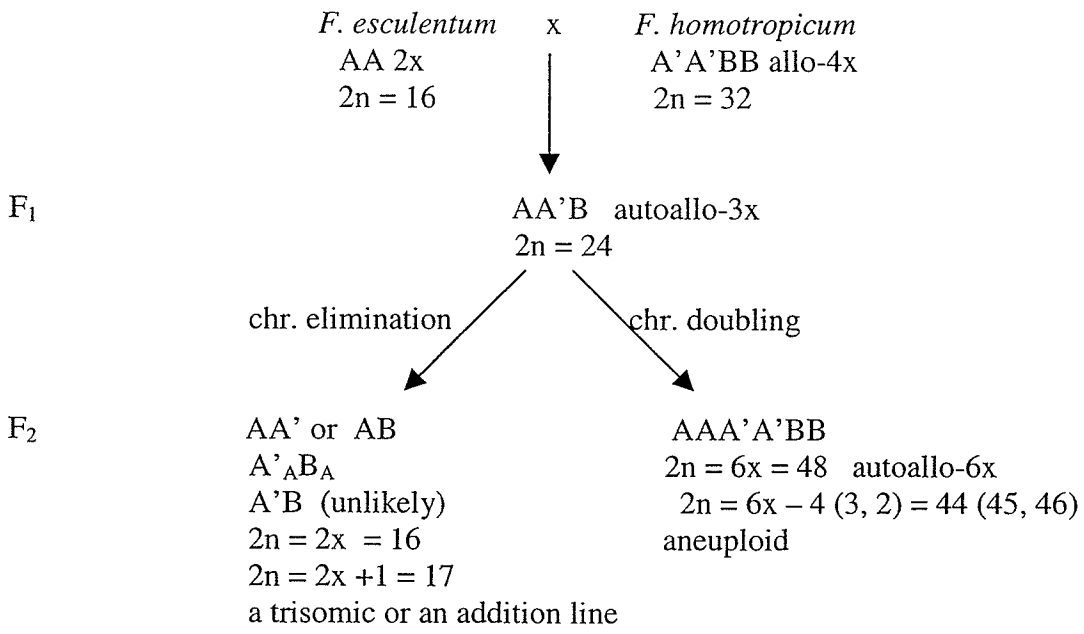

Figure 3.2.2. Variation of ploidy in interspecific hybrids between *F. esculentum* (2n = 2x = 16) and *F. homotropicum* (2n = 4x = 32).

Aneuploid plants (2n = 2x +1 = 17). One plant with 17 chromosomes (2n = 2x +1 =17) was found in this study. It is not clear whether the extra chromosome in this aneuploid line was derived from *F. homotropicum* or *F. esculentum.* If two sets of chromosomes and the extra chromosome came from the same genome, the line would be a trisomic; otherwise it could be an addition line. In either case, the aneuploid line will be valuable for future genetic studies including mapping and the location of genetic markers on the extra chromosome.

CONCLUSIONS

Variation in chromosome numbers was observed in the interspecific hybrids and successive generations between *F. esculentum* and *F. homotropicum* tetraploid. Fertility was restored by either chromosome doubling or chromosome elimination in the triploid hybrid, resulting in hexaploid (2n = 48), diploid (2n = 16), and aneuploid (2n = less or

more than 48) in the F_2 progeny. One aneuploid line ($2n = 2x + 1 = 17$) was developed which will provide valuable material for future genetic studies.

MANUSCRIPT 3

Interspecific Hybridization between *Fagopyrum tataricum* (L.) Gaertn and *F. esculentum* Moench

ABSTRACT

The objective of this study was to improve the success of interspecific hybridization between the two cultivated buckwheat species, *Fagopyrum tataricum* and *F. esculentum*. Three sterile hybrids were produced from the interspecific cross of the two species from a total of 111 ovules in tissue culture. One of the three hybrids was a triploid derived from unreduced female gamete of *F. tataricum*. Chromosome doubling did not restore fertility. One sterile hybrid was obtained by crossing the amphihexaploid with a fertile hybrid of *F. esculentum* / *F. homotropicum* 2x. One bridge hybrid was produced by combining two single crosses with *F. homotropicum* 4x as a bridge species. Pollen viability of this bridge hybrid was 18.5% compared to the hybrid at 1.7% from the direct cross of *F. tataricum* / *F. esculentum*, and pollen viability increased to 21.8% after the bridge hybrid was crossed with a self-compatible line that had a similar genetic background to *F. esculentum*. These results demonstrate the possible approaches to restore hybrid fertility by recurrent backcrossing of the hybrids with the self-compatible line from the cross of *F. esculentum* / *F. homotropicum*. It is expected that the final products would retain some of the *F. tataricum* genome through recombination prior to chromosome elimination and offer the possibility in buckwheat improvement.

INTRODUCTION

Efforts to hybridize the two cultivated buckwheat species, *Fagopyrum tataricum* and *F. esculentum*, began as early as 1951 (Morris, 1951). This cross was initiated in an attempt to combine the self-compatibility from *F. tataricum* and the larger seed size with looser seed coat from *F. esculentum*. *Fagopyrum tataricum* has stable yield, frost tolerance, and the feature of self-compatibility expressed in its homostyle flower. The seed has high rutin content with benefits for human health. However, the seed size is small and the

flour has a bitter taste. The tight seed coat makes dehulling impossible. The very small flowers (Figure 3.3.1A: left) make emasculation and pollination a challenge for buckwheat breeders. *Fagopyrum esculentum* is the more widely cultivated species. The large size seed, loose seed coat, non-bitter flour taste, and larger flower size than *F. tataricum* (Figure 3.3.1A: right) for hybridization have resulted in breeding effort focused on *F. esculentum* rather than of *F. tataricum*. However, the yield of *F. esculentum* is unstable. The causes of instability of yield in *F. esculentum* has been identified as inefficient pollination associate with inefficient pollinators (Kreft, 1983, Namai, 1990; Fesenko, N. V., 1990), frost susceptibility (Armstrong, 1999; Campbell, 1997; and Gaberscik, et al., 1986), moisture and high temperature stresses (Fesenko, N. V., 1990 and Lachanov, et al., 1989), and nutritional deficiency due to high number of flowers and inefficient distribution of assimilate to developing grains (Sugawara, 1960; Kreft, 1986; Ruszkowski, 1990; and Fesenko, 1990). A comparative study conducted by Ruszkowski (1980) found no significant difference between *F. esculentum* and *F. tataricum* in plant vigor, dry and green matter accumulation, assimilation area, photosynthetic rate, root development and most yield components. The only difference of the two species was the percentage of seed set, suggesting that self-incompatibility alone may be the cause of low seed set in *F. esculentum*. It is of interest to note that, in any program for the improvement of *F. esculentum* using *F. tataricum* as a germplasm source, there also exists the possibility of improving *F. tataricum* with very little extra effort (Campbell, 1997).

Since Morris (1951) started to hybridize the two cultivated buckwheat species, numerous breeders have repeated this cross at both the diploid and tetraploid level, assisted by ovule rescue *in vitro* (Hirose et al, 1995; Samimy 1991; Samimy et al. 1996; Chen 1998; Woo 1998; and Fesenko, I.N., et al. 2001). In addition, somatic hybridization has been carried out through protoplast fusion by Lachmann, et al. (1994). The results of these efforts demonstrate the difficulty of producing successful interspecific hybrids. Samimy et al. (1996) reported one hybrid from 263 rescued ovules. The hybrid was sterile and could not be maintained. However, no effort has been made to cross the two species through different strategies such as multiple crosses or bridge crosses to overcome the difficulty of the cross, which are common approaches in other crops as in wheat,

Brassicas, tobacco, potato, and cotton (Hadley and Openshaw, 1980; Vyas et al., 1995; and Vroh Bi et al., 1999). In this study, we attempted to hybridize the two cultivated species through three strategies, direct cross, multiple cross, and bridge cross to produce interspecific hybrids and improve hybrid viability.

MATERIALS AND METHODS

Plant materials and the types of crosses. Three species were used as parents. The two cultivated species, *F. tataricum* and *F. esculentum*, are diploid (2n = 2x = 16). *Fagopyrum homotropicum* is a wild species and has both diploid (2n = 2x = 16) and tetraploid (2n = 4x = 32) forms. The following types of crosses were made:

1. The direct cross: *F. tataricum* (B770198)/ *F. esculentum* pin (509);
   In this cross, *F. tataricum* was used as the female and crossed with *F. esculentum* pin plants.
2. The multiple cross: the amphiploid //*F. esculentum* (Drullet) / *F. homotropicum* 2x (K950818-3);
   The amphiploid was produced by colchicine treatment from the hybrid described in 1.
3. The bridge cross: *F. tataricum* (B770198) / F. *homotropicum* 4x (K950818-2) // *F. esculentum* (509) / *F. homotropicum* 4x (K950818-2);
   In this cross, *F. homotropicum* 4x was used as a bridge species connecting the two cultivated species.
4. The backcross: hybrid from described in 3 / X990133;
   X990133 was a self-compatible line derived from a convergent cross:
   BM94999.1/Drullet // Drullet /K950818-3 /// BM94999.1*2 / K950818-3

BM94999.1 and Drullet are two accessions of *F. esculentum* and K950818-3 is an accession of *F. homotropicum* 2x. Therefore, this convergent cross was presumed to have 75% of the genetic background of *F. esculentum*.

Hybrid development from the direct cross. Flowers to be used as females were emasculated one day before pollination or the early morning prior to pollination. The ovule rescue technique was applied to prevent embryo abortion approximately 7-10 days after pollination. The ovules of *F. esculentum* / *F. homotropicum* 2x were cultured in a half strength MS medium (Murashige and Skoog, 1962) and developed directly into plantlets. The remainder of the crosses required three culture media before the hybrid development was complete, including the media of initiation; shoot development, and root formation. The media composition and culture conditions have been described in a previous study (Wang and Campbell, 1998) (Appendix 6.1).

Chromosome doubling. Cotton balls were soaked in an aqueous solution of colchicine 0.1% (w/v) and were then placed on the axillary buds of the juvenile plants from stem cuttings. The treatment was maintained for 24 h in a mist chamber. The misting system was set for 6 seconds at 32 min. intervals for the first hour and then for 4 seconds at 64 min. intervals for the remaining 23 h.

Chromosome counts. Plant root tips and flower buds were used for chromosome counts. Root tips were collected, pretreated in a solution of 0.15 ml/ml water of bromonapthalene for 1.5 h at room temperature, and were then fixed in Farmer's fixative (95% ethanol: acetic acid = 3:1) for at least one day. The root tips were hydrolyzed in 3N HCl at 60°C for 20 min., and stained with 2% acetocarmine, and then squashed in a drop of 45% acetic acid on a slide for observation under a light microscope (x1250). Young flower buds were collected at meiosis, stained by acetocarmine, squashed, and observed under a light microscope.

Pollen viability. Pollen samples were stained with an iodine solution containing 0.05% iodine and 0.1% potassium iodide. Approximately 500 to 900 pollen grains were examined in each hybrid. Pollen grains that were large and stained blue were scored as viable, while small and non-stained pollen grains were scored as inviable.

RESULTS AND DISCUSSION

The direct cross of F. tataricum / F. esculentum. Three hybrids were obtained from the 111 rescued ovules. The hybrids had the homostyle flower of the *F. tataricum* with flower size intermediate between the two parents (Figure 3.3.1A). Two of three plants were tall with few branches and the third plant was short with many branches. The plants were sterile and pollen viability was as low as 1.7% (Table 3.3.1).

Cytological examination of the root tip cells determined that two hybrids had 16 chromosomes, presumably the result of fusion of n = 8 gametes from each parent. One hybrid that was short with many branches had 24 chromosomes. Within the karyotype, 16 small chromosomes could be distinguished from eight large chromosomes (Figure 3.3.2A and Table 3.3.1). It is known that the average size of chromosomes of *F. esculentum* is much larger than of *F. tataricum* (Fesenko, I. N. et al., 2001); therefore the 16 small chromosomes probably resulted from an unreduced 2n gamete from the female parent *F. tataricum* which combined with a normal n = 8 gamete from *F. esculentum.* The occurrence of unreduced 2n gametes from both male and female parents has been widely documented in the plant kingdom as one of contributing mechanisms to polyploidy (Poehlman and Sleper, D. A. 1995). Unreduced gametes have occurred in the process of interspecific hybridization in Brassicas (Gundimeda et al., 1992 and Vyas et al., 1995) and later generations of hybrids between *Triticum aestivum* and *Agropyron cristatum* (Chen et al., 1992).

Table 3.3.1. Cytological characters and pollen viability of hybrids from the four types of crosses, original cross, multiple cross, bridge cross, and backcross, among *F. tataricum*, *F. esculentum*, and *F. homotropicum*.

Cross	Number Hb/OR	Chromosome Number	Chromosome Composition	Pollen viability %
1. Original cross:				
F. t / F. e	3/111			1.7
		16	8 t + 8 e = 16	
		24	16 t + 8 e = 24	
		↓		
Amphiploid		48	32 t + 16 e = 48	
2. Multiple cross:				
(*F.t / F.e*) amphiploid // *F.e / F.h* 2x	1/17	48	(24 t + 16 e) + 8 e/h = 48	1.4
3. Bridge cross:				
F.t / F.h 4x // *F.e / F.h* 4x	1/1	43	8 t + 8e + 32h = 48	18.5
		↓		
4. Backcross				
F.t / F.h 4x // *F.e / F.h* 4x /// X990133	2/51	36	unknown	21.8

F. e: *F. esculentum*; *F. t*: *F. tataricum*; *F. h*: *F. homotropicum*; Hb/OR: hybrids/ovules rescued.

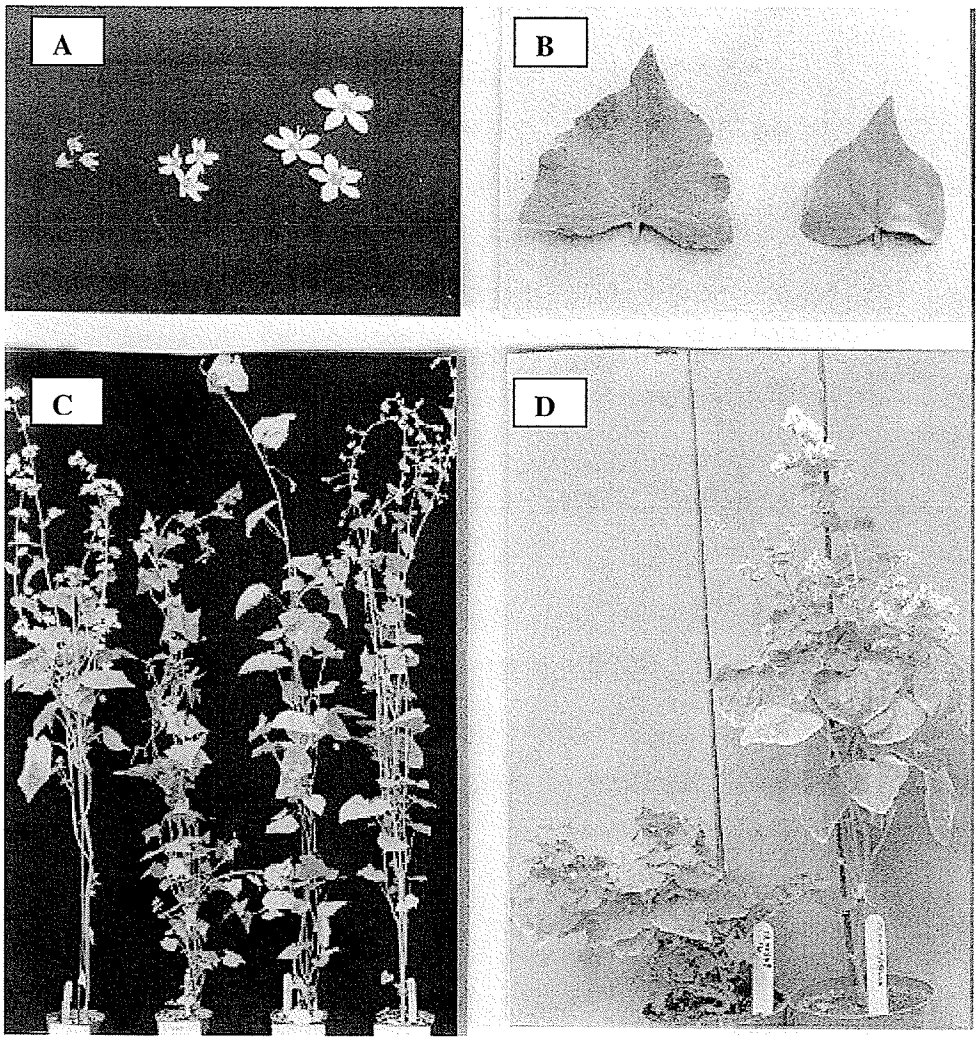

Figure 3.3.1. Characteristics of hybrids and their parents. A: flowers. Left-*F. tataricum*, right-*F. esculentum*, and middle-hybrid; B: leaves of *F. tataricum* / *F. esculentum* hybrid. Left-after chromosome doubling, right-before chromosome doubling; C: From left to right – *F. esculentum*, *F. homotropicum* 4x, the bridge hybrid, and *F. tataricum*; D: hybrid plants. Left–the amphihexaploid of *F. tataricum* / *F. esculentum*, right–the multiple hybrid from amphiploid // *F. esculentum* / *F. homotropicum* 2x.

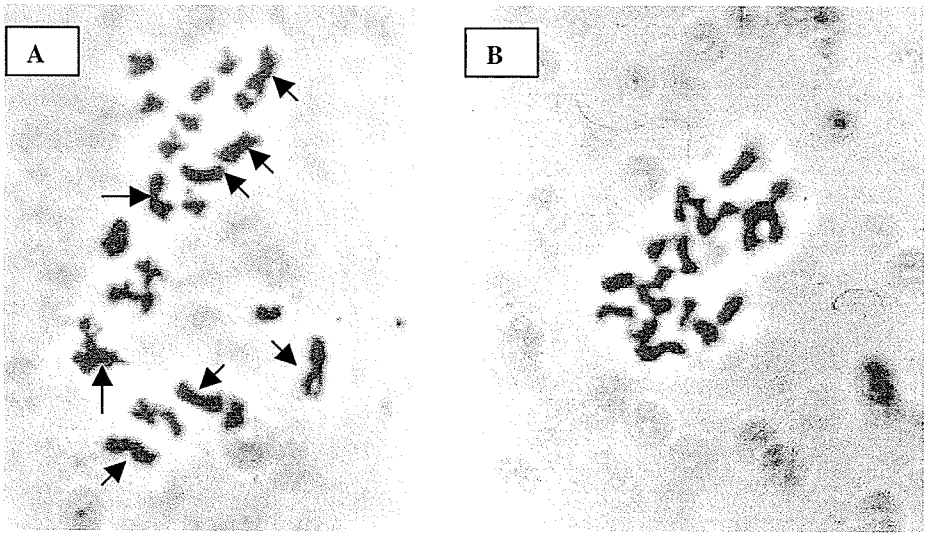

Figure 3.3.2. The chromosomes of hybrids. A: 24 chromosomes of *F. tataricum* / *F. esculentum* at mitosis with eight large chromosomes from *F. esculentum* (arrows) and 16 small chromosomes from *F. tataricum*; B: 18 chromosomes of the bridge hybrid / self-compatible line X990133 at meiosis.

Restoration of hybrid fertility by chromosome doubling. After the hybrids were treated with colchicine to induce chromosome doubling, the leaves that emerged subsequently were thicker and darker green compared with non-treated hybrids (Figure 3.3.1B). The hybrid fertility was not restored by the colchicine treatment. One of two doubled hybrids died before the chromosome numbers were determined. The other hybrid plant that originally had 24 chromosomes was determined to have 48 chromosomes after treatment, confirming that it was an amphihexaploid with 16 large chromosomes derived from *F. esculentum* and 32 small chromosomes from *F. tataricum* (Table 3.3.1). If the hybrid sterility is caused by the feature of chromosome pairing between the parental genomes, chromosome doubling should restore fertility (Hadley and Openshaw, 1980). Chromosome doubling did not restore the fertility of the hybrid between *F. tataricum* and *F. esculentum*; therefore the sterile hybrids might be caused by other factors such as genic or cytoplasmic sterility.

Multiple cross of the amphihexaploid // F. esculentum / F. homotropicum 2x. Backcrossing is a common method to restore non-chromosomal hybrid sterility. We

backcrossed both the original hybrid and amphihexaploid with both *F. tataricum* and *F. esculentum* without successful restoration. One hybrid was developed from the multiple cross between the amphihexaploid and a fertile hybrid of *F. esculentum* / *F. homotropicum* 2x. The success of this multiple hybrid might be due to the fact that the two parents had the same type of self-compatible homostyle flower.

The new hybrid was taller than the original amphihexaploid hybrid with many flower clusters (Figure 3.3.1D), but was still sterile with low pollen viability (Table 3.3.1). This hybrid had the same number of chromosomes (48) as the original amphihexaploid. However, the number of the small chromosomes from the *F. tataricum* parent was reduced from 32 to 24. The loss of 8 chromosomes was presumably compensated by the addition of 8 large chromosomes from either *F. esculentum* or *F. homotropicum* or both parents of the cross (Table 3.3.1). The multigenomic origin of the chromosomes (Table 3.3.1: 24 chromosomes from *F. tataricum* + 16 from *F. esculentum* + 8 from both *F. esculentum* and *F. homotropicum* or either one) would disrupt chromosome pairing at meiosis, resulting in sterility in the new hybrid.

It is possible that fertility could be restored if the resulting multiple hybrid was repeatedly crossed with the fertile hybrid of *F. esculentum* / *F. homotropicum* 2x. This process is similar to the hybrid being backcrossed with *F. esculentum* with the distinction that the recurrent parent is self-compatible and contains the genetic background of *F. homotropicum*, a bridge species between *F. tataricum* and *F. esculentum*. It is predicted that the small *F. tataricum* chromosomes would be eliminated and the final hybrid would similar to the *F. esculentum* and *F. homotropicum* with some desirable genes or chromosomal fragments from *F. tataricum* as a result of genetic recombination resulting among three sets of chromosomes. These hybrids would achieve the same fertility as fertile hybrids between *F. esculentum* and *F. homotropicum*.

The bridge crosses of F. tataricum / F. homotropicum 4x // F. esculentum / F. homotropicum 4x. Bridge crosses are another approach used to hybridize two distant species that cannot be crossed directly or are very difficult to cross. This strategy has

been employed in the economically important species of Brassicas, tobacco (*Nicotiana*), cotton (*Gossypium*), and potato (*Solanum*) (Hadley, 1980; Vyas et al., 1995; and Vroh Bi et al., 1999). In bridge crosses, one or two bridge species are used to connect the two targeted species. In this study, we chose *F. homotropicum* as a bridge species since it has the same breeding systems as *F. tataricum* and is cross compatible with both species (Wang and Campbell, 1998).

One hybrid was produced from the crosses between the two single crosses, *F. tataricum* / *F. homotropicum* 4x and *F. esculentum* / *F. homotropicum* 4x. This hybrid was vigorous and distinct from all three parental species (Figure 3.3.1C 3rd plant). It had self-compatible homostyle flower and pollen viability was greatly improved at 18.5%, compared with the hybrids from the direct cross of *F. tataricum* / *F. esculentum* at 1.7% (Table 3.3.1). The chromosome number of this hybrid (43) was less than the expected chromosome number of 48 (Table 3.3.1), indicating that five chromosomes were lost during crossing. Based on cytological analysis, at least five small chromosomes of *F. tataricum* were retained, suggesting that some of the lost chromosomes were from either *F. esculentum* or *F. homotropicum.*

Overcoming the sterility of the bridge cross hybrid using the self-compatible line X990133. The self-compatible line, X990133, was developed from a convergent cross and was presumed to contain 75% the genetic background of *F. esculentum.* Therefore it could be used as a recurrent parent that is similar to *F. esculentum* in backcrosses to restore hybrid fertility. The two hybrids produced from the cross between the bridge hybrids and X990133 were vigorous with large leaves and flowers. The pollen viability was increased from 18.5% to 21.8% (Table 3.3.1) compared to the bridge hybrid. The chromosome number of the hybrids was reduced from 43 to 36 (Fig 3.3.2B at meiosis), indicating seven chromosomes were lost during crossing.

It is not easy to predict the chromosome composition after several cycles of crossing. However, the situation is similar to the multiple cross discussed above. After several cycles of backcrossing, the small *F. tataricum* chromosomes would be eliminated

gradually with eventual restoration of fertility in the hybrid and the possibility of some contribution from the *F. tataricum* genome as a result of recombination.

This study described several approaches to hybridize between the two cultivated species, *F. tataricum* and *F. esculentum*, through direct cross of the two species and indirect crosses using *F. homotropicum* as the bridge species. It is anticipated expected that hybrid fertility will be gradually restored through recurrent crossing of the sterile hybrids with self-compatible lines derived from the cross between *F. esculentum* and *F. homotropicum*. The significance of this study is not only in the production of interspecific hybrids, but also in the demonstration of different strategies of hybridization in *Fagopyrum* genus. These results support the use of multiple hybridization methods in difficult crosses instead of the traditional hybridization method that only relies on direct crosses.

MANUSCRIPT 4

S^h and S_c - Two Complementary Dominant Genes That Control Self-Compatibility in Buckwheat

ABSTRACT

There are three flower types in buckwheat (*Fagopyrum* spp.), self-compatible homostyly and self-incompatible heterostylic pin and thrum. The inheritance of self-compatibility was investigated in four interspecific crosses between self-compatible *F. homotropicum* and self-incompatible *F. esculentum.* The data from F_2 populations of the interspecific hybrids, the BC_1F_1 to *F. esculentum* pin, F_3 lines derived from F_2 homostyly plants, and crosses of pin x homostyly among individual plants selected from the progenies of interspecific hybrids supported the genetic control of self-compatibility of *F. homotropicum* by two complementary dominant genes that relate to self-incompatibility of *F. esculentum.* The first gene has three alleles S, S^h, and s corresponding to the three types of flowers in which thrum is dominant to homostyly and pin, and homostyly is dominant to pin as $S>S^h>s$ as proposed by Woo et al. The second gene, identified in this study, is represented by S_c for self-compatibility, and has two alleles with S_c producing the homostyly that is dominant to s_c producing the heterostylic pin. The genotype of *F. homotropicum* is proposed as $S^hS^hS_cS_c$, whereas there are two groups of genotypes possible in *F. esculentum*, ssS_cS_c, ssS_cs_c or sss_cs_c producing the pin flower type and SsS_cS_c, SsS_cs_c or, Sss_cs_c producing the thrum phenotype. The alleles at the first gene locus are fixed in both pin and thrum flower types.

INTRODUCTION

The high nutrient value of cultivated common buckwheat (*Fagopyrum esculentum*) as human food is compromised by unstable seed yield mainly due to its dependence on insects and wind for pollination and its susceptibility to frost (Campbell, 1997). A plant improvement program has been initiated using self-pollinated buckwheat derived from interspecific hybridization between *F. esculentum* and a wild species *F. homotropicum* (Campbell, 1995; Wang and Campbell, 1998; and Woo et al., 1999a). *Fagopyrum*

homotropicum is a self-compatible species collected from the Southwest China by Ohnishi (1995) that is closely related to *F. esculentum* (Yasui and Ohnishi, 1998a).

Fagopyrum species have two reproductive types: homostyly, in which the styles and stamens are the same height and plants are self-compatible, and heterostyly, which corresponds to self-incompatibility with pin and thrum dimorphic flowers. The pin flower has long styles that protrude above the stamens, whereas thrum flowers have short styles located below the stamens. The two flower types are produced on different plants and fertilization occurs only between pin and thrum flowers. *Fagopyrum esculentum*, one of the two cultivated buckwheat species, is self-incompatible and expresses heterostyly.

Inheritance studies of heterostyly in buckwheat have demonstrated that the S allele coding for thrum is dominant to the s allele coding for pin, located at the single gene locus S (Althausen, 1908; Dahlgren, 1922; Eghis, 1925; Garber and Quisenberry, 1927; and Saknarov, 1946). A supergene complex has been proposed by Sharma and Boys (1961) as in *Jepsonia parryi* (Ornduff, 1970) and in *Primula viscose* (Ernst, 1936). The model assumed that the complex consists of five subgenes corresponding to stylar incompatibility, pollen incompatibility, style length, pollen size, and stamen height, respectively. Fesenko N. N. (1985, 1986, and 1989) has suggested the similar model with three subgenes coding for style length, stamen length, and pollen size. Homostyle flowers that resulted from X ray treatment in a positive thrum plant had the same reaction as thrum flower implied that the subgene for style length had changed only (Sharma and Boys, 1961). However, this model was not finalized because the experiment could not rule out the possibility of self-incompatibility and self-compatibility under different genetic control. Similar observations were reported by Marshall (1969). He selected self-compatible lines with reduced style length in *F. esculentum*. The F_2 data from his preliminary crosses among the inbred lines indicated the influence of at least two modifying genes that controlled the difference in the length of the style.

The previous inheritance studies on self-compatibility were mostly conducted in common buckwheat based on homostyle mutants from distyly. Fesenko, N.N. (1989) suggested

inheritance of homostyly is under control by the modifier genes or polygenes. However, due to the difficulty of interspecific hybridization, there is limited information regarding inheritance of homostyly and its relationship with heterostyly based on interspecific hybrids. Inheritance of self-compatibility in different *Fagopyrum* species might be controlled by different genetic systems, for example, mutated homostyly in *F. esculentum* and homostyly in the self-compatible species *F. homotropicum*.

The segregation ratio of 3 homostyly: 1 pin in the F_2 hybrids between *F. esculentum* pin and *F. homotropicum* (Campbell, 1995; Wang and Campbell, 1998) supported a single gene model with homostyly being dominant to the heterostylic pin. Woo et al. (1999a) proposed that the S gene controlling heterostyly and homostyly has multiple alleles S, S^h, and s with the S^h allele coding for homostyly. Heterostylic thrum is dominant to homostyly, whereas homostyly is dominant to heterostylic pin $S>S^h>s$. The F_1 thrum plants, from their cross *F. esculentum* thrum / *F. homotropicum*, were backcrossed to pin plants of *F. esculentum* and a segregation ratio of 5 homostyly: 5 thrum was obtained. However, there was one exceptional plant that produced pin flowers in the BC_1F_1. This occurrence could not be explained by the proposed single gene model. In interspecific crosses between homostyly *F. homotropicum* and heterostylic pin of *F. esculentum*, we observed that segregation ratios of 3:1 in F_2 populations and 1:0 or 1:1 in crosses of pin x homostyly among the interspecific progenies did not always fit a single gene theory. Fesenko et al (1998) obtained hybrids from the cross between *F. esculentum* thrum and *F. homotropicum*. The F_1 were either homostyly or heterostylic thrum. Homostylic plants segregated into 3 homostyly: 1 pin that fitted one gene model. However, when F_1 self-incompatible thrum plants were randomly mated with pin plants, the progeny had three types: thrum, homostyly, and pin, in which pin plants were not expected in one gene model. Based on this observation, they conclude that homostyly of *F. homotropicum* is not allelism of heterostyly of *F. esculentum*. Therefore, the objective of the present study was to determine the inheritance of self-compatibility in *F. homotropicum* based on interspecific hybrids between *F. homotropicum* and *F. esculentum*.

MATERIALS AND METHODS

The interspecific crosses were made between heterostylic pin flowers of three *F. esculentum* accessions (BM940364, BM94999.1 and X980088) and homostyle flowers of two *F. homotropicum* accessions (K980854 and K980855). Successful hybrid development was achieved in four crosses by means of ovule rescue as described by Wang and Campbell (1998). Variable success in ovule culture resulted in a different number of hybrids being obtained from the four crosses. These hybrids could be genetically distinct due to the heterogeneity of cross-pollinating *F. esculentum.* Backcrosses between the F_1 hybrids and *F. esculentum* pin plants and crosses between homostyly and pin plant flowers among the interspecific hybrids were made. Segregation patterns of homostyly to pin were recorded for the F_2 of the interspecific hybrids, the BC_1F_1, the F_3 lines derived from F_2 homostyle plants, and F_1 progeny resulting from crosses between pin x homostyly plants among the progenies of the interspecific hybrids. Chi-square analysis was used to test the goodness of fit to expected one and two gene segregation ratios. All plants were grown in a greenhouse maintained at a minimum temperature of 22°C with natural light supplemented to a 16 h / 8 h day light photoperiod.

RESULTS AND DISCUSSION

The F_2 and BC_1F_1 generation. Fifty-two F_1 hybrids from individual embryos of four crosses were obtained through ovule rescue. All F_1 plants were homostyly, indicating that self-compatible homostyly is dominant to self-incompatible heterostylic pin. Segregation patterns for both one gene (3:1) and two genes (9:7) in the 8 F_2 populations are possible if two complementary dominant genes control the self-compatibility (Table 3.4.1). S^h and S_c represent corresponding homostyle alleles and s and s_c represent the corresponding heterostylic pin at two gene loci. When one of the two gene pairs is heterozygous e.g. $S^hsS_cS_c$ in the F_1, the segregation ratio of homostyly: heterostylic pin would be 3:1 in the F_2 population and 1:1 in the BC_1F_1 progeny. When both genes are heterozygous $S^hsS_cs_c$ in the F_1 hybrid, the F_2 generation would produce a 9:7 homostyly: pin ratio, while the BC_1F_1 generation would be 1:1, 3:5 or 1:3 when the F_1 is backcrossed to plants in different genotypes of the heterogeneous population *F. esculentum* (Figure 3.4.1). In the four crosses (Table3.4.1), three out of eight F_2 populations had a

segregation ratio of 9:7. Among four backcrosses, three had a 3:1 segregation ratio in F_2 and a 1:1 segregation ratio in BC_1F_1. One of the backcrosses had a 9:7 segregation ratio in F_2 and the BC_1F_1 segregation fitted both 1:1 and 3: 5 ratios. The χ^2 values of the 3:5 ratio was 0.05 and of the 1:1 ratio was 2.17, suggesting that the 3:5 ratio was a closer fit (Table3.4.1). This would support a two gene model (Figure 3.4.1).

Segregation ratios of the F_3 lines derived from F_2 homostyle plants. The two populations with a segregation ratio of 3:1 in the F_2 generation had segregation ratios of 1:0 or 3:1 in the F_3 from the F_2 homostyle lines (Table 3.4.2). One population with a segregation ratio of 9:7 in the F_2 generation showed segregation ratios of 1:0, 3:1, or 9:7 (Table 3.4.2). These results confirm the control of self-compatibility by two complementary dominant genes. The segregation ratios of 1:0, 3:1 and 9:7 in the F_3 lines correspond to the genotypes $S^hS^hS_cS_c$, $S^hsS_cS_c$ and $S^hsS_cs_c$ in the F_2 homostyle plants.

Segregations of pin x homostyly crosses among the progeny of the interspecific hybrids. Unlike the original interspecific cross between *F. esculentum* and *F. homotropicum*, the F_1 progeny from the crosses between heterostylic pin and homostyly among the progenies of the interspecific hybrids have 5 possible types of segregation ratios i.e. 1:0, 1:1, 3:1, 3:5, and 1:3 (Table 3.4.4), because both heterostylic pin and homostyle plants can be heterozygous at both loci. The segregation of the progeny from 6 out of the 14 pin x homostyly crosses did not fit either the segregation ratio of 1:0 or 1:1, the only two ratios for one dominant gene model (Table 3.4.3). One cross, S980013, produced a closer fit to a 3: 5 ratio than a 1:1 ratio. These results support the model of two complementary dominant genes controlling the self-compatibility in buckwheat.

Table 3.4.1. Segregation ratios (homostyly: pin) of the F_2 and the BC_1F_1 in interspecific hybrids between *F. homotropicum (h)* and *F. esculentum (e)*.

Parents		F_2			BC_1F_1				Expected	
			χ^2			χ^2				
F. h	*F. e*	Ob	3:1	9:7	Ob	1:1	3:5	1:3	F_2	BC_1F_1
K980854	BM94999.1	95:22	2.40	29.59**	12:6	2.00			3:1	1:1
K980854	X980088	69:13	3.66	25.93**					3:1	
K980854	BM940364	53:30	5.50*	1.95					9:7	
		82:22	0.82	21.58**					3:1	
		82:32	0.57	11.39**	27:21	0.75			3:1	1:1
K980855	BM940364	58:28	2.62	4.38*	5:11	2.25			3:1	1:1
		51:33	9.14**	0.68					9:7	
		58:40	13.07**	0.34	18:28	2.17	0.05	4.90*	9:7	3:5
										1:1

df = 1: P = 0.05, *χ^2 = 3.84; P = 0.01, **χ^2 = 6.64; Ob: observation.

Table 3.4.2. Segregation ratios (homostyly: pin) of the F_3 lines derived from F_2 homostyle plants in three individual hybrids.

Cross	F_2 ratio	F_3 Ob	χ^2 1:0	χ^2 3:1	χ^2 9:7	Expected	P
X980088	3:1	56:20	-	0.07	9.39**	3:1	0.80-0.90
x		31:7	-	0.88	9.91**	3:1	0.60-0.70
K980854		26:0	0	-	-	1:0	1.00
		30:12	-	0.29	3.93*	3:1	0.60-0.70
K980854	3:1	17:5	-	0.06	3.95*	3:1	0.80-0.90
x		20:0	0	-	-	1:0	1.00
BM94999.1		24:0	0	-	-	1:0	1.00
		32:5	-	2.60	13.75**	3:1	0.10-0.20
K980855	9:7	26:0	0	-	-	1:0	1.00
x		35:14	-	0.33	4.59*	3:1	0.60
BM940364		10:13	-	12.19**	1.52	9:7	0.20-0.30
		15:15	-	10.00**	0.48	9:7	0.50
		29:0	0	-	-	1:0	1.00
		31:11	-	0.03	5.26*	3:1	0.80-0.90

df = 1: P = 0.05, *χ^2 = 3.84; P = 0.01, **χ^2 = 6.64; Ob: observation.

Figure 3.4.1. Possible segregation patterns of crosses between *F. esculentum* **pin** (p) and *F. homotropicum* homostyly (h)

1. ssS_cS_c x $S^hS^hS_cS_c$

↓

F_1	$S^hsS_cS_c$	x	*F. esculentum* **p**	BC_1F_1
	↓		ssS_cS_c	1h:1p
F_2	3h:1p		ssS_cs_c	1h:1p
	↓		sss_cs_c	1h:1p
F_3	1h:0p, 3h:1p			

2. sss_cs_c x $S^hS^hS_cS_c$

↓

F_1	$S^hsS_cs_c$	x	*F. esculentum* **p**	BC_1F_1
	↓		ssS_cS_c	1h:1p
F_2	9h:7p		ssS_cs_c	3h:5p
	↓		sss_cs_c	1h:3p
F_3	1h:0p, 3h:1p, 9h:7p			

3. ssS_cs_c x $S^hS^hS_cS_c$

↓

F_1	$S^hsS_cS_c$	$S^hsS_cs_c$
	↓	↓
F_2	3h:1p	9h:7p

Gene notation:
Two alleles at the first locus: S^h – homostyly; and s – pin;
Two alleles at the second locus: S_c – homostyly; s_c – pin.

Figure 3.4.2. Possible segregation patterns from crosses between *F. esculentum* thrum (t) and *F. homotropicum* homostyly (h).

1. SsS_cS_c x $S^hS^hS_cS_c$

↓

F_1	$S^hsS_cS_c$	$SS^hS_cS_c$	x	*F. esculentum* **pin (p)**	BC1F1
	1 h	1 t		ssS_cS_c	1t:1h
	↓			ssS_cs_c	1t:1h
F_2	3h:1p			sss_cS_c	1t:1h
	↓				
F_3	1h:0p, 3h:1p				

2. Sss_cs_c x $S^hS^hS_cS_c$

↓

F_1	$S^hsS_cs_c$	$SS^hS_cs_c$	x	*F. esculentum* **p**	BC_1F_1
	1h	1 t		ssS_cS_c	1t:1h
	↓			ssS_cs_c	**4t:3h:1p**
F_2	9h:7p			sss_cS_c	**2t:1h:1p**
	↓				
F_3	1 h:0p, 3h:1p, 9h:7p				

3. SsS_cs_c x S^hS^hScSc

↓

F_1	$S^hsS_cs_c$	$S^hsS_cS_c$	$SS^hS_cS_c$	$SS^hS_cs_c$
	1h	1h	1t	1t
	↓	↓		
F_2	9h:7p	3h:1p		

Gene notation:
Three alleles at the first locus: S – thrum; S^h – homostyly; and s – pin;
Two alleles at the second locus: S_c – homostyly; s_c – pin.

Fagopyrum homotropicum and F. esculentum genotypes. The homostyly allele was designated S^h in the one gene model by Woo, et al. (1999a), in which the relationships of three types of flowers was described as $S>S^h>s$, i.e. thrum was dominant to homostyly, and homostyly dominant to pin. Our two gene model maintains S^h as the homostyly allele at the first gene locus and proposes S_c, a self-compatible allele, at the second gene locus. The proposed genotype of *F. homotropicum* would be $S^hS^hS_cS_c$ as *F. homotropicum* is pure breeding for homostyly and heterozygosity at either of the loci would result in the production of homozygous recessive plants with a pin phenotype. *Fagopyrum esculentum* pin and thrum plants, on the other hand, can have more than one genotype if the first locus is fixed as homozygous recessive ss for the pin genotype and heterozygous Ss for the thrum genotype. This model explains why homostyly is not produced in the crosses between heterostylic thrum and pin flowers in *F. esculentum*. At the second locus, the S_c allele coding for homostyly is dominant to the allele s_c coding for pin. Therefore, the proposed genotypes for *F. esculentum* pin plants are ssS_cS_c, ssS_cs_c, or sss_cs_c and for *F. esculentum* thrum plants are SsS_cS_c, SsS_cs_c, or Sss_cs_c.

In the interspecific hybrid between *F. esculentum* and *F. homotropicum*, all of the possible four homostyly genotypes, $S^hS^hS_cS_c$, $S^hsS_cS_c$, $S^hS^hS_cs_c$ and $S^hsS_cs_c$, will be produced in the resulting progeny if the F_1 hybrids are heterozygous at both gene loci (Figure 3.4.1 and Figure 3.4.2).

The two gene model explains the 9:7 ratio in the F_2 (Figure 3.4.1 and Figure 3.4.2) and the ratios of 3:5 or 1:3 (Figure 3.4.1), 4:3:1 (thrum: homostyly: pin) or 2:2:1 (thrum: homostyly: pin) (Figure 3.4.2) in the BC_1F_1, which appear in the different crosses. The ratio of 4:3:1 fits the backcross result of 5 thrum: 5 homostyly: 1 pin obtained by Woo, et al. (1999a) and provides an explanation for the one pin plant that could not be accommodated in their single gene model. In the two gene model, this backcross would

Table 3.4.3. Segregation ratios (homostyly: pin) of the F_1 progenies from the pin x homostyly crosses in progeny of *F. esculentum* x *F. homotropicum*.

Cross	Ob	χ^2 1:0	1:1	3:1	3:5	1:3	Expected ratio (probability)		
S980008	20:0	0	-	-	-	-	1:0 (1.00)		
S980010	21:0	0	-	-	-	-	1:0 (1.00)		
X990073	27:0	0	-	-	-	-	1:0 (1.00)		
X990095	29:0	0	-	-	-	-	1:0 (1.00)		
X980014	17:13	-	0.53	5.38*	4.70*	16.04**	1:1 (0.30-0.50)		
X990089	15:13	-	0.14	6.86**	3.09	12.19**	1:1 (0.70)	3:5 (0.10-0.05)	
X990116	13:13	-	0	8.67**	1.73	8.67**	1:1 (1.00)	3:5 (0.10-0.20)	
S980005	19:6	-	6.76**	0.01	15.81**	34.68**	3:1 (0.90)		
X990142	18:6	-	6.00*	0	14.00**	32.00**	3:1 (1.00)		
X990161	20:9	-	4.17*	0.56	12.25**	29.90**	3:1 (0.40-0.50)		
S980013	15:27	-	3.43	34.57**	0.06	2.57	3:5 (0.80-0.90)	1:3 (0.10)	1:1 (0.05-0.10)
S000001	18:34	-	4.92*	45.23**	0.18	2.56	3:5 (0.50)	1:3 (0.30)	
X990129	4:15	-	6.37*	29.49**	2.19	0.16	1:3 (0.70)	3:5 (0.10-0.20)	
X00175	6:16	-	4.55*	26.73**	0.98	0.06	1:3 (0.80-0.90)	3:5 (0.30)	

df = 1: P = 0.05, *χ^2 = 3.84; P = 0.01, **χ^2 = 6.64; Ob: observation

Table 3.4.4. Possible segregation patterns (homostyly: pin) in the F_1 progenies from pin x homostyly crosses in buckwheat.

Genotypes of the pin parents	Genotypes of the homostyly parents			
	$S^hS^hS_cS_c$	$S^hS^hS_cs_c$	$S^hsS_cS_c$	$S^hsS_cs_c$
$S^hS^hs_cs_c$	1:0	1:1	1:0	1:1
$S^hss_cs_c$	1:0	1:1	3:1	3:5
ssS_cS_c	1:0	1:0	1:1	1:1
ssS_cs_c	1:0	3:1	1:1	3:5
sss_cs_c	1:0	1:1	1:1	1:3

be derived from the cross of $SS^hS_cs_c$ x ssS_cs_c (Figure 3.4.2). The two gene model also confirms the result of Fesenko et al. (1998) that three phenotypes of flowers were observed in the progeny from randomly mating F_1 thrum with pin plants.

The application of inheritance of self-compatibility in the breeding of self-pollinated buckwheat. The transfer of self-compatibility from *F. homotropicum* to *F. esculentum* involves crosses between homostyle and heterostylic species. In the present study, only heterostylic pin flowers were used in the interspecific crosses. Due to the differences between pin and thrum in morphology and genetics, the pin plant has advantages over thrum plants in a crop improvement program. From a morphological viewpoint, thrum flowers are more difficult to cross than pin flowers as the styles of thrum are imbedded among stamens. It is also more difficult to distinguish homostyly from thrum in the small buckwheat flowers (diameter about 3-4 mm). From a genetic viewpoint, the thrum flowers are heterozygous, carrying the pin allelomorph in *F. esculentum* and possibly both pin and homostyly alleles after the hybrid (thrum) plants are crossed with pin (Figure 3.4.2), to produce the genotypes Ss and SS^h at the first locus. The resulting cross between thrum and homostyle plants would produce progeny with all three types of flowers, requiring additional selection. The use of only pin type plants in crosses to homostyly plants eliminates the thrum phenotype and facilitates selection of homostyly from pin as the style of the pin flower is conspicuous above the stamens.

Further study. The present study proposes that two types of segregation ratios, 3:1 and 9:7, in the F_2 correspond to the genotypes for one and two heterozygous gene(s) in the F_1 population. Since we are proposing that the first locus of *F. esculentum* is fixed as either ss (pin) or Ss (thrum), a segregation ratio of 3:1 in the F_2 infers that the first gene is heterozygous in the population (Figure 3.4.1). On the other hand, if the F_2 has a segregation ratio of 9:7, there may be two types of heterozygous homostyly plants among the progeny, i.e. heterozygosity at both loci or heterozygosity at one of the two loci. Aii et al. (1999) developed a co-dominant SCAR (sequence characterized amplified region) marker for self-compatibility from an interspecific hybrid between *F. homotropicum* and *F. esculentum* pin. The marker should be linked with the first gene, as the F_2 segregation ratio was a 3:1. Therefore, a marker could be developed for the second gene in a population containing the two heterozygous genes (F_2 9:7) using the co-dominant marker of Aii et al. (1999) to distinguish the first gene from the second gene.

MANUSCRIPT 5

Inheritance of Seed Shattering in Interspecific Hybrids between *Fagopyrum esculentum* and *F. homotropicum*

ABSTRACT

Fagopyrum esculentum, a cultivated buckwheat species, is resistant to seed shattering. Its yield, however, is dependent on insects and wind to cross-pollinate the two types of flowers, pin and thrum, which occur on different plants. A new self-pollinated buckwheat has been developed from interspecific hybridization between *F. esculentum* and the wild species, *F. homotropicum*, which is self-compatible but is prone to seed shattering. Therefore, understanding the genetic control of shattering is of considerable importance in the development of the self-pollinated buckwheat. Seed shattering was evaluated in four interspecific crosses between *F. esculentum* and *F. homotropicum*. The F_1 hybrids were shattering and the F_2 populations from individual F_1 hybrids segregated in the ratios of 3:1, 9:7, and 27:37 indicating that shattering was controlled by three complementary dominant genes with shattering dominant to non-shattering. The different segregation ratios in the F_2 populations may have been caused by heterogeneity in *F. esculentum* due to cross-pollination within the population. The genotype of *F. homotropicum* is homozygous dominant that prevents the occurrence of non-shattering genotypes, while *F. esculentum* must be homozygous recessive at a minimum of one locus that prevents production of shattering genotypes. The present study provides a clear understanding of how shattering can be produced from crosses between two non-shattering parents. Non-shattering plants, on the other hand, can be obtained from either crossing or selfing shattering plants.

INTRODUCTION

Fagopyrum esculentum has better flour quality, larger seed size, and the seeds are easier to dehull compared to the other cultivated species *F. tataricum*. However, yield instability is a significant problem in *F. esculentum* production. One of main reasons is the dependence on wind and insect vectors to cross-pollinate the two types of flowers, pin

type flowers with long styles and thrum with short styles that are grown on separate plants. *Fagopyrum homotropicum* with homostyle self-compatible flowers is a wild species, collected by Ohnishi (1995) from the Southwest of China. A self-pollinated buckwheat hybrid was developed from interspecific hybridization between *F. esculentum* and *F. homotropicum* (Campbell, 1995 and Wang and Campbell, 1998). The self-pollinated buckwheat has the desirable characteristics from *F. esculentum*, with the self-compatibility of *F. homotropicum*. However, seed shattering, an agronomical undesirable trait, was also introduced from *F. homotropicum* into the self-pollinated buckwheat.

Little is known about the inheritance of seed shattering in buckwheat because the two cultivated buckwheat species are both resistant to shattering. Fesenko et al. (1998) and Ohnishi (1999) reported that seed shattering is controlled by a single gene in which shattering is dominant to non-shattering, using the crosses between the non-shattering cultivated buckwheat *F. esculentum* and its shattering ancestor *F. esculentum* ssp. *ancestrale*. The segregation ratio of 3:1 (Wang and Campbell, 1998 and Fesenko et al., 1998) has been reported in the F_2 populations in the interspecific hybrids between non-shattering *F. esculentum* and shattering *F. homotropicum*. However, the bulked F_2 populations might not reflect the real inheritance patters due to the heterogeneity of *F. esculentum* from cross-pollinating. Therefore, the objective of this study was to determine the inheritance of seed shattering in the hybrids between *F. esculentum* and *F. homotropicum* based on individual F_2 populations that were derived from individual F_1 plants.

MATERIALS AND METHODS

Crossing program. The heterostylic pin flowers of three accessions of *F. esculentum*, BM940364, BM94999.1 and X990088 were crossed with the homostyle flowers of two *F. homotropicum* accessions, K980854 and K980855. Three crosses (K980854 / MB940999.1, K980854 / BM940364, and K980855 / BM940364) were made using *F.*

homotropicum as the female, while one cross (X980088 / K980854) used *F. esculentum* pin as the female.

Population development. A total of 52 F_1 hybrids were obtained from individual embryos using ovule rescue *in vitro* (Wang and Campbell, 1998). Theoretically, these hybrids were genetically distinct due to the heterogeneity of cross-pollinated *F. esculentum* parents. Eight F_2 populations each derived from a single F_1 hybrid plant were selected from the four crosses.

The segregation of the F_2 populations fitted three ratios of 3:1, 9:7, and 27:37 shattering to non-shattering, corresponding to the monogenic, digenic and trigenic segregation. To confirm the F_2 segregations, 28 F_3 lines were produced from the F_2 shattering plants with self-compatibility, consisting of 14 lines from a F_2 trigenic population, eight lines from a F_2 digenic population, and six lines from a F_2 monogenic population (Table 3.5.3).

Confirmation of seed shattering in self-incompatible pin plants. Two flower types, self-compatible homostyly and self-incompatible heterostylic pin, were produced in the F_2 and F_3 populations as the homostyly is dominant to pin. In order to observe seed shattering, seeds were obtained from pin plants by hand pollination using pollen from randomly selected homostyly plants.

Measurements and analysis. The number of shattering and non-shattering plants was recorded from the F_1 hybrids, the F_2 and the F_3 populations. Plants that expressed shattering were bagged until harvest. Seeds that did not drop with hand disturbing were considered as non-shattering. Chi-square analysis was used to test the fit of the observed segregation to the expected ratios.

All plants were grown in the greenhouse of a minimum temperature of 22°C with natural light supplemented to a 16 h / 8 h day/night light photoperiod.

RESULTS AND DISCUSSION

Genetic control of seed shattering in the F_1 and F_2 generations. Fifty-two F_1 hybrids from individual embryos were obtained through ovule rescue in four interspecific crosses. All the F_1 plants expressed shattering, indicating that shattering is dominant to non-shattering. The analysis of the eight F_2 populations showed that three populations fitted a ratio of 3:1, two populations fitted a ratio of 9:7, and three populations fitted a ratio of 27:37 shattering to non-shattering (Table 3.5.1). These results support a model of three complementary genes SH_1, SH_2, and SH_3 controlling shattering. Genotypes which are homozygous recessive at a minimum of one locus e.g. $SH_1\_SH_2\_sh_3sh_3$ produce the non-shattering phenotype, while genotypes with a dominant allele at each of the three loci $SH_1\_SH_2\_SH_3\_$ produce the shattering phenotype. As there were no non-shattering plants in the two accessions of *F. homotropicum*, the genotype of *F. homotropicum* can be represented $SH_1SH_1SH_2SH_2SH_3SH_3$. The distinct segregation ratios in the F_2 populations can be explained by heterogeneity at two of the shattering gene loci in the *F. esculentum* populations and homozygous recessive alleles fixed at one locus to prevent shattering in this species (Table 3.5.2).

The 28 F_3 lines derived from the F_2 shattering plants were divided into three groups according to the one, two, and three gene segregation ratios (Table 3.5.3). Of the 14 F_3 lines from the F_2 population with the three gene segregation ratio of 27:37, two lines segregated shattering to non-shattering 1:0, one line 3:1, four lines 9:7 and seven lines 27:37, corresponding homozygous dominant, one, two, and three heterozygous gene(s) respectively, in the F_2 population. Of the 8 F_3 lines from the F_2 population with the two-gene segregation ratio of 9:7, four lines had a segregation ratio shattering to non-shattering of 3:1 and four lines had the ratio of 9:7. These ratios indicate that one gene (3:1) and two genes (9:7) were heterozygous in their respective F_2 plants. Homozygous dominant plants were not identified in this group, possibly due to the fact that the sample size was small and theoretically only one out of 9 shattering plants should be homozygous dominant in the F_2 population. The third group, with a single gene of ratio of 3:1 in the F_2 population, had segregation ratios of 1:0 and 3:1 in its F_3 lines,

confirming that only one gene was segregating in this population. The results from all the F_3 lines agreed with the results of the segregation ratios found in the F_2 generation.

Seed shattering in the two accessions of F. homotropicum and in F. esculentum spp. ancestrale studied by Ohnishi (1999). In this study, the F_2 populations from the parent *F. homotropicum* accession K980854 showed one and two gene segregation ratios, while the three F_2 populations from the other parent *F. homotropicum* accession K980855 showed a three gene segregation ratio (Table 3.5.1). The genotype of *F. homotropicum* must be homozygous dominant at the three loci, as the segregation of recessive alleles at any one of the three gene loci would produce non-shattering plants in this species. These different segregation patterns were possibly caused by different parental genotypes of *F. esculentum*, a cross-pollinating species (Table 3.5.2).

Fesenko et al. (1998) and Ohnishi (1999) reported that seed shattering in *F. esculentum* spp. *ancestrale* (the ancestor of *F. esculentum*), was controlled by one complete dominant gene based on the segregation ratio of 3:1 found in the F_2 populations from the crosses between *F. esculentum* and *F. esculentum* spp. *ancestrale*. According to our model, one gene segregation ratio would be due to the genotype of *F. esculentum*, $sh_1sh_1SH_2SH_2SH_3SH_3$, if F. *homotropicum* and *F. esculentum* spp. *ancestrale* have the common shattering genes (Table 3.5.2). However, it is also possible that the two species have the distinct shattering genes.

Table 3.5.1. Segregation of shattering (S) and non-shattering (NS) plants in the F_2 populations from interspecific crosses Between *F. homotropicum* and *F. esculentum.*

Parents		Observed S: NS	χ^2				
F. homotropicum	*F. esculentum*		3:1	9:7	27:37	Expected	P
K980854	BM94999.1	101:24	2.24	30.61**	76.41**	3:1	0.10-0.20
K980854	X980088	47:35	13.67**	0.04	7.70**	9:7	0.80-0.90
K980854	BM940364	85:28	0	16.53**	50.56**	3:1	1
		48:30	7.54**	0.89	11.98**	9:7	0.30-0.40
		83:22	0.92	22.18**	58.49**	3:1	0.30-0.40
K980855	BM940364	32:54	65.50**	12.67**	0.87	27:37	0.40-0.50
		30:54	69.14**	14.39**	1.44	27:37	0.20-0.30
		39:59	64.78**	10.78**	0.23	27:37	0.60-0.70

df = 1: P = 0.05, *χ^2 = 3.84; P = 0.01, **χ^2 = 6.64.

Table 3.5.2. Proposed genotypes of *F. homotropicum* and *F. esculentum* coded to the segregation patterns of the F_2.

Proposed genotypes		Segregation of F_2
F. homotropicum	*F. esculentum*	S: NS
$SH_1SH_1SH_2SH_2SH_3SH_3$	$sh_1sh_1SH_2SH_2SH_3SH_3$	3:1
	$sh_1sh_1sh_2sh_2SH_3SH_3$	9:7
	$sh_1sh_1SH_2SH_2sh_3sh_3$	9:7
	$sh_1sh_1sh_2sh_2sh_3sh_3$	27:37

S: shattering; NS: non-shattering.

Table 3.5.3. Segregation of shattering (S) and non-shattering (NS) plants in F_3 lines derived from F_2 shattering plants from interspecific crosses between *F. homotropicum* and *F. esculentum.*

Parents			# F_3 lines	Observed				
F. homotropicum	*F. esculentum*	F_2	pooled	S	NS	Expected	χ^2	P
K980855	BM940364	27:37	2	54	1	1:0	0.02	0.90
			1	31	11	3:1	0.03	0.80-0.90
			4	99	80	9:7	0.06	0.80-0.90
			7	104	155	27:37	0.44	0.50
K980854	X980088	9:7	4	113	42	3:1	0.36	0.50-0.60
			4	114	96	9:7	0.33	0.50-0.60
K980854	BM94999.1	3:1	3	57	2	1:0	0.07	0.80-0.90
			3	67	19	3:1	0.39	0.50-0.60
Total			28					

df = 1: P = 0.05 χ^2 = 3.84; P = 0.01 χ^2 = 6.64.

Table 3.5.4 Examples of shattering buckwheat progenies resulted from the crosses between two non-shattering parents.

Cross	Number of plants	Number of Shattering	Number of Non-shattering
T990002	16	16	0
X000196	40	40	0
X990135	41	19	22
T990015	16	7	9
T000029	16	4	12
X000195	23	15	8
X990180	10	8	2

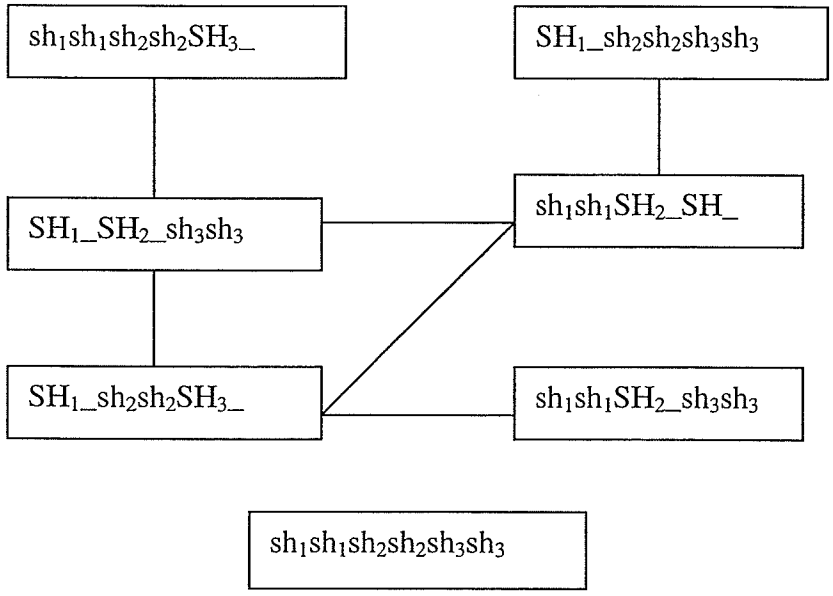

Figure 3.5.1. Possible crosses between two non-shattering buckwheat parents that result in seed shattering.
Shattering phenotypes will result from two non-shattering genotypes that are connected by a line.

Breeding for non-shattering character in self-pollinated buckwheat. The present study supports the model of three complementary genes controlling seed shattering in *F. homotropicum*. According to this model, shattering sometimes may occur in the progenies produced from two non-shattering parents (examples in Table 3.5.4) if they have different alleles at the same locus (Figure 3.5.1). On the other hand, non-shattering genotypes could be produced by crossing between two shattering parents or by selfing shattering plants that are heterozygous dominant. As a result of this complex inheritance, breeding non-shattering self-pollinated buckwheat will require large populations for effective selection. In addition, shattering is strongly linked with self-compatible homostyly flowers (data not shown), and a large population will be required to select for recombinant genotypes in which the linkage is broken.

Seed shattering in buckwheat is associated with the presence of an abscission layer across the pedicel (Oba, et al., 1998). This study observed three types of pedicels in buckwheat. The first type has no visible ring around pedicel. The second type has visible ring around the pedicel with no restriction. Seed shattering does not occur in these two types. The third type has a clearly visible abscission layer with a restriction of the pedicel, the point at which the seed shatters. Attempts to identify physical markers that are linked to shattering genotypes, for example, the types of pedicels, has not been successful. Molecular marker assisted selection (MAS) has the potential to increase the efficiency of crossing and selection based on genotypes rather than phenotypes.

MANUSCRIPT 6

The Genetic Control of Winged Seed in Intraspecific and Interspecific Hybrids of *Fagopyrum homotropicum* Ohnishi

ABSTRACT

The inheritance of winged seeds was studied in intraspecific hybrids of the species *F. homotropicum* and in interspecific hybrids between *F. homotropicum* and *F. esculentum*. Based on the segregation ratios of the F_2, F_3, and BC_1F_1 generations, two complementary genes W_1 and W_2 control winged seed, with winged seed being dominant to non-winged. In *F. homotropicum*, it is proposed that the genotype of the winged seed type is represented as $W_1W_1W_2W_2$. In the non-winged genotype of *F. homotropicum*, one locus is fixed as homozygous dominant and the other locus as homozygous recessive $W_1W_1w_2w_2$ or $w_1w_1W_2W_2$ which leads to an inheritance pattern appearing as a single gene locus. To produce non-variable non-winged phenotype of *F. esculentum*, one of two loci must be fixed due to cross-pollination of this species, producing the three possible genotypes, represented as $W_1W_1w_2w_2$, $W_1w_1w_2w_2$, and $w_1w_1w_2w_2$. These genotypes of *F. esculentum* produce the different segregation patterns observed in the interspecific hybrids between *F. homotropicum* and *F. esculentum*, acting as either one or two gene inheritance, respectively.

INTRODUCTION

Fagopyrum homotropicum is a wild species of the *Fagopyrum* genus. The 19 populations of this species in collection have been studied by morphological, cytological, and isozyme analysis (Asano, 1998). *Fagopyrum homotropicum* is closely related to cultivated common buckwheat *F. esculentum* (Ohnishi, 1995) and has the favorable characteristics of self-compatibility, green testa, and high rutin content, therefore it has been widely used to improve *F. esculentum* through interspecific hybridization.

The cultivated *F. esculentum* is cross-pollinating with a heteromorphic sporophytic self-incompatibility system. It normally has non-winged seed that is triangular in shape and

no protrusion of the hull at the junction where the three sides meet. Winged forms of *F. esculentum* are also known to occur occasionally. *Fagopyrum homotropicum* has both non-winged seed as well as winged seed with prominent protrusion of the hull at the junction of the three sides. Winged seed is a desirable character in wild species as it increases seed dispersal. However, this character is undesirable in a cultivated crop as it reduces seed density and causes problems with cleaning equipment. Winged seed became a problem in buckwheat breeding programs when the interspecific hybrids between *F. esculentum* and *F. homotropicum* produced winged seed type (Campbell, 1995). Very limited information was found in published literature regarding the inheritance of winged seed, both in buckwheat and in other plants. In a previous report (Wang and Campbell, 1998), the segregation in a bulked F_2 generation from interspecific hybrid between *F. homotropicum* and *F. esculentum* was 192 winged to 96 non-winged plants, which was not an obvious inheritance pattern. In the same type of interspecific cross, Zeller and Hsam (2001) reported a ratio of 9:7 winged: non-winged in a F_2 population, suggesting that two complementary genes control the winged seed trait. However, the inheritance of this character should be determined by a number of F_2 populations that are separated by F_1 single plants, as *F. esculentum* might have variable genotypes due to cross-pollinating. To develop more efficient procedures to eliminate winged seeds in buckwheat breeding programs, the objectives of this study were: 1) to determine the inheritance of winged seed based on populations that were derived from the single F_1 plants in interspecific hybrids between *F. homotropicum* and *F. esculentum*; and 2) to compare the inheritance patterns between intraspecific hybrids within *F. homotropicum* and interspecific hybrids from *F. homotropicum* and *F. esculentum*.

MATERIALS AND METHODS

Plant material. The winged parents came from three accessions of *F. homotropicum*, K980854, K950818-1, and K950818-3. *Fagopyrum esculentum* and another two accessions of *F. homotropicum* K980855 and K950818-3 were used as non-winged parents. The accession of K950818-3 produced plants with both winged seeds and non-winged seeds that were separately grown and purified in the greenhouse.

Hybrid production. Interspecific hybrids between *F. homotropicum* and *F. esculentum* were produced, using ovule rescue *in vitro* as described in a previous study (Wang and Campbell, 1998). Intraspecific hybrids within *F. homotropicum* were produced through emasculation and pollination within two days without ovule rescue *in vitro*.

The F_1 hybrid plants were randomly selected for backcrossing and planting to produce F_2 populations individually or bulked. Each F_2 population produced from a F_1 single plant was represented by approximately 100 plants in the interspecific hybrids and 70 plants in intraspecific hybrids unless the single F_1 plant did not produce enough seeds. One bulked F_2 population consisted of four F_1 hybrids from the cross between *F. homotropicum* (an accession K950818-1) and *F. esculentum* (an accession BM940364). The F_3 lines used for study were derived from the F_2 plants with winged seeds. Parental plants were grown for comparison with each generation of hybrid production.

Measurements and analysis. The segregation of winged and non-winged seeds was recorded for each generation. Chi-square analysis was used to test the goodness of fit to expected one and two gene ratios. All plants were grown in a greenhouse with a minimum temperature of 22°C and natural light supplemented to a 16/8 h day/night photoperiod.

RESULTS AND DISCUSSION

Segregation of intraspecific hybrids within F. homotropicum. In reciprocal crosses, all of the 107 F_1 hybrid plants had the winged seed phenotype (Table 3.6.1), indicating winged seed was dominant to non-winged with no maternal effect. The segregation of all 10 F_2 populations fitted a 3:1 ratio winged: non-winged (Table 3.6.1). The segregation of four BC_1F_1 populations in K980854 and one pooled BC_1F_1 population in K950818-3 fitted a ratio of 1:1 when the F_1 was backcrossed to the non-winged parents (Table 3.6.1). Those results supported a single gene control of the character.

Segregation patterns of interspecific hybrids between F. homotropicum and F. esculentum. The segregation patterns of two F_2 populations from the hybrid population 2 and 5 fitted a 3:1 ratio winged: non-winged, their corresponding BC_1F_1 fitted a 1:1 ratio and the F_3 fitted the ratio of either 1:0 or 3:1 winged: non-winged (Table 3.6.2), supporting a model of one gene control of the character. The other two F_2 populations from the hybrid populations 9 and 10 had a 9:7 ratio winged: non-winged and the BC_1F_1 corresponding from hybrid 10 had a ratio of 3:5 winged: non-winged (Table 3.6.2). The remaining three BC_1F_1 from the hybrid populations 4, 7, and 11 without planting F_2 populations had either the ratio of 3:1 or 3:5 winged: non-winged. These results supported a model of two gene control of the character (Table 3.6.3). The one bulked F_2 population from four F_1 hybrids that had been reported in the previous study (Wang and Campbell, 1998) had 192 plants with winged seeds and 96 plants with non-winged seeds (Table 3.6.2), comfirming a ratio of 21:11 winged: non-winged (Table 3.6.2).

Based on these results, a two gene model is proposed in which two complementary genes control the character for winged seed, with the winged dominant to non-winged (Table 3.6.3).

Proposed genotypes of F. homotropicum. In *F. homotropicum*, the genotype for winged seed plant is proposed as $W_1W_1W_2W_2$ and for non-winged seed plant as $W_1W_1w_2w_2$. The F_2 and BC_1F_1 from the cross between the two genotypes would be expected to segregate with ratios of 3:1 winged: non-winged and 1:1 respectively (Table 3.6.3). The genotype of $W_1w_1w_2w_2$ is unlikely to occur in the non-winged genotype of *F. homotropicum*. If this genotype were crossed with the winged genotype $W_1W_1W_2W_2$, there would be two genotypes in the F_1, $W_1W_1W_2w_2$ and $W_1w_1W_2w_2$, producing both 3:1 and 9:7 winged: non-winged segregation ratio in the F_2 generation (Table 3.6.3). Only the 3:1 winged: non-winged segregation ratio was observed in the 10 populations of F_2 and all of the BC_1F_1 with non-winged parents had 1:1 ratios (Table 3.6.1) supporting the proposed model.

Table 3.6.1. Segregation patterns of plants with winged (w) and non-winged (nw) seeds in the F_2, F_3, and BC_1F_1 generations in intraspecific crosses within *F. homotropicum.*

Parent				Number	Observed				
w	nw	Cross	Generation	plant/population	w	nw	Expected	χ^2	P
K980854	K980855	nw x w	F_1	51	51	0	1:0	0	1.00
			F_2	2	99	29	3:1	0.38	0.60
			BC_1F_1 nw	2	49	52	1:1	0.09	0.80
			BC_1F_1 w	2	99	0	1:0	0	1.00
		w x nw	F_1	23	23	0	1:0	0	1.00
			F2	2	137	41	3:1	0.37	0.60
			BC_1F_1 nw	2	52	48	1:1	0.16	0.70
			BC_1F_1 w	2	80	0	1:0	0	1.00
K950818-1	K980855	nw x w	F_1	2	2	0	1:0	0	1.00
			F_2	2	107	32	3:1	0.29	0.60
		w x nw	F_1	1	–	–	–	–	–
K950818-3	K950818-3	nw x w	F_1	13	13	0	1:0	0	1.00
			F_2	2	74	19	3:1	1.04	0.30
			BC_1F_1 nw	6	16	24	1:1	1.6	0.20
		w x nw	F_1	17	17	0	1:0	0	1.00
			F_2	2	54	20	3:1	0.16	0.70
			BC_1F_1 w	6	15	15	1:1	0	1.00

BC_1F_1 nw/w: F_1 backcrossed with the non-winged or winged parent; df = 1: P = 0.05 χ^2 = 3.84; P = 0.01 χ^2 = 6.64.

Table 3.6.2. Segregation patterns of plants with winged (w) and non-winged (nw) seeds in the F_2, F_3, and BC_1F_1 generations in the interspecific crosses between *F. homotropicum* x *F. esculentum.*

F. homotropicum	Hybrid			Observed		F_2, F_3, χ^2				BC_1F_1, χ^2				
Accession (w)	No.	Generation	Number lines	w	nw	1:0	3:1	9:7	21:11	1:1	1:3	3:5	Expected	P
K980854	2	F_2		61	15		1.12	17.81**					3:1	0.30
x	2	F_3	1	46	0	0.00							1:0	1.00
nw	2	F_3	7	213	74		0.09	37.64**					3:1	0.80
	5	F_2		92	25		0.82	23.82**					3:1	0.40
	5	BC_1F_1nw		22	22					0.00			1:1	1.00
	5	F_3	4	108	1	0.04							1:0	0.80-0.90
	5	F_3	2	36	6		2.57	14.82**					3:1	0.10-0.20
	7	BC_1F_1nw		10	21					3.90*		0.36	3:5	0.60
	4	BC_1F_1nw		11	35					12.52**	0.03		1:3	0.80-90
K950818-1	10	F_2		17	14		6.72**	0.03					9:7	0.80-0.90
X	10	BC_1F_1nw		10	19					2.79		0.11	3:5	0.70
nw														
	9	F_2		75	51		16.10**	0.55					9:7	0.50
	bulk	F_2		192	96		10.67**	12.7**	0.14				21:11	0.8-70
	11	BC_1F_1nw		5	30					17.86**	2.14		1:3	0.10-20

BC_1F_1nw: F_1 backcrossed with non-winged parent; pop.: population; df = 1: P= 0.05, *χ^2 = 3.84; P= 0.01, **χ^2 = 6.64.

Table 3.6.3. Proposed genotypes of *F. homotropicum* and *F. esculentum* and the expected segregation ratios (w:nw) deduced from the phenotypes and segregation patterns in intraspecific and interspecific crosses.

Cross	Generation	Winged genotype	Non-winged genotype		
			$W_1W_1w_2w_2$	$W_1w_1w_2w_2$	$w_1w_1w_2w_2$
F. homotropicum x *F. homotropicum*	F_1	$W_1W_1W_2W_2$	w ($W_1W_1W_2w_2$)		
	F_2		3:1		
	BC_1F_1 (BC with *F. h* nw)	$W_1W_1W_2w_2$	1:1		
	BC_1F_1 (BC with *F. h* w[a])	$W_1W_1W_2w_2$	w		
F. homotropicum x *F. esculentum*	F_1	$W_1W_1W_2W_2$	w ($W_1W_1W_2w_2$)	w ($W_1W_1W_2w_2$+$W_1w_1W_2w_2$)	w ($W_1w_1W_2w_2$)
	F_2		3:1	21:11	9:7
	BC_1F_1 (BC with *F. e* nw)	$W_1W_1W_2w_2$ (F_1)	1:1	1:1	1:1
	BC_1F_1 (BC with *F. e* nw)	$W_1w_1W_2w_2$ (F_1)	1:1	3:5	1:3

BC_1F_1 nw: F_1 backcrossed with the non-winged; [a]: backcrossed between the F_1 $W_1W_1W_2w_2$ hybrid with the winged parent $W_1W_1W_2W_2$

Proposed genotypes for F. esculentum. The non-winged seed plants in *F. esculentum* would have the three possible genotypes, $W_1W_1w_2w_2$, $W_1w_1w_2w_2$, and $w_1w_1w_2w_2$ (Table 3.6.3), due to the heterogeneity of the cross-pollinating populations. However, in the proposed model, one of two loci is fixed as homozygous recessive to explain the absence of winged phenotypes.

When the winged genotype $W_1W_1W_2W_2$ in *F. homotropicum* was crossed with *F. esculentum* on a random basis, two possible genotypes, $W_1W_1W_2w_2$ and $W_1w_1W_2w_2$ would be produced in the F_1, in a ratio of 1:1, with the corresponding F_2 ratios of 3:1 and 9:7 winged: non-winged (Table 3.6.3). The results from our study had segregation patterns that fitted ratios of 3:1 and 9:7 winged: non-winged in the F_2 generation from single F_1 hybrids (Table 3.6.2), supporting the two gene model. A segregation ratio in the F_2 reported by Zeller and Hsam (2001) infers that the non-winged genotype of *F. esculentum* in their cross probably was $w_1w_1w_2w_2$ (Table 3.6.3). Ratios of both 3:5 and 1:3 winged: non-winged would be possible in the BC_1F_1 plants in the two gene model (Table 3.6.3). If the F_1 population was bulked consisting of the genotypes 1:1 $W_1W_1W_2w_2$: $W_1w_1W_2w_2$, the corresponding F_2 would segregate into the ratio of 21:11 winged: non-winged (Table 3.6.3). This model explains the results from the bulked F_2 population that fitted the ratio of 21:11 (Table 3.6.2) that could not be explained in the previous study (Wang and Campbell, 1998).

Comparison of non-winged genotypes between F. homotropicum and F. esculentum. In our two gene model, the *F. homotropicum* genotype for the non-winged phenotype is fixed as homozygous dominant at one locus and as homozygous recessive at the other locus to ensure that the winged seed phenotype cannot arise. This distribution of alleles makes *F. homotropicum* appear to be controlled by a single gene. In *F. esculentum*, one locus is fixed as homozygous recessive, but the second locus could have any combination of dominant or recessive alleles resulting in the variable segregation ratios in the F_2 and BC_1F_1 generations from different hybrids (Table 3.6.3).

Explanation of winged seeds in F. esculentum based on the two gene model. There has not been an explanation for the occasional occurrence of the winged seed phenotype in cultivated *F. esculentum*. In cultivated *F. esculentum*, non-winged seed genotypes could mutate to the winged seed genotypes at the fixed loci, for example, $W_1W_1w_2w_2$ or $W_1w_1w_2w_2$ to $W_1W_1W_2w_2$ or $W_1w_1W_2w_2$ even though the natural mutation rate is very low.

Variable crossability of accessions within F. homotropicum. The non-winged parent K980855 was easily crossed with the winged parent K980854, showing high seed set and no seed abortion. However, the crosses between K980855 and the winged parent K950818-1 were difficult to produce and had low seed set and a high rate of seed abortion. This difference could reflect the relative genetic distance between the different populations. The accessions K950818-3 was of particular interest. This accession has both winged and non-winged plants. The hybrid seeds from the cross between winged and non-winged plants were not abort after fertilization, but the seed set was much lower than the rest of the intraspecific crosses, especially in the backcrosses to the non-winged parent in which seed set from pollinated flowers was lower than 14% (data not shown). The reason for this low success rate is not clear, as it cannot be attributed to genetic distance within a single accession.

4.0 GENERAL DISCUSSION AND CONCLUSIONS

Inter-Relates Information Presented in Each Manuscript of the Thesis

Development of interspecific hybrid between the two cultivated species, F. esculentum and F. tataricum using the bridge species F. homotropicum. The production of interspecific hybrids between *F. esculentum* and *F. tataricum* through direct cross has been difficult to achieve (Fesenko, I. N. et al., 2001), even though it would be desirable to combine several characters from the two species. This thesis described the production of hybrids between *F. homotropicum* and the two cultivated species, *F. esculentum* and *F. tataricum*, providing the bridge crosses for crossing the two cultivated species. Interspecific hybrids between the two cultivated species were successful by using *F. homotropicum* as a bridge species. The bridge hybrids were vigorous and expressed greatly improved pollen viability compared with the hybrids from the direct cross between the two cultivated species. Although the hybrids were sterile, it is expected that fertility would eventually be restored by successively backcrossing the hybrids with the self-compatible lines derived from the cross between *F. esculentum* and *F. homotropicum* diploid.

Chromosome variation in interspecific hybrids. In this thesis, interspecific hybrids were produced from the three species, including *F. homotropicum* / *F. esculentum*, *F. homotropicum* / *F. tataricum*, *F. esculentum* / *F. tataricum*, as well as multiple crosses. Chromosome variation was observed in these hybrids. An unreduced female gamete from *F. tataricum* occurred in the cross between *F. esculentum* and *F. tataricum*. Spontaneous chromosome doubling restored hybrid fertility in the cross between *F. esculentum* and *F. homotropicum* tetraploid. In the fertile progeny of the same cross, chromosome elimination from the genome of both parents was observed. Chromosome elimination also occurred in the multiple and bridge crosses among the three species. In addition, aneuploids were frequently observed in the interspecific hybrids.

Variable genetic segregation patterns resulting from heterogeneity of F. esculentum. In the three genetic studies on self-compatibility, seed shattering, and winged seed, a common problem was encountered in interpreting the complex segregation patterns in the

F_2 and BC_1F_1. The reason was that *F. esculentum* maintained a high level of heterogeneity due to the outcrossing. It was necessary to consider the possible genotypes of *F. esculentum* before the inheritance of these characters could be interpreted.

There are two parts to the interpretation. First, one locus has to be fixed to explain why the expression of certain characters does not vary in *F. esculentum*. One of two loci has to be fixed as "ss" in the pin flower and "Ss" in the thrum flower to prevent production of the homostyle flower type. One of three loci has to be fixed, as homozygous recessive "shsh" to maintain the non-shattering character and prevent the occurrence of shattering and one of two loci has to be fixed as homozygous recessive "ww" to prevent production of the winged phenotype.

The second part is that the phenotype of one character in *F. esculentum* is produced by more than one genotype, resulting in different segregation patterns in the F_2 and BC_1F_1 generations. This was illustrated in all three genetic studies. In the future, it is recommended that inheritance studies using *F. esculentum* as a parent in interspecific hybrids should analyze F_2 populations derived from individual F_1 hybrids rather than from a bulked F_1 population. In addition, the sufficient numbers of F_2 populations should be produced to allow all possible segregation patterns to be tested.

Even though the heterogenic nature of *F. esculentum* makes inheritance studies challenging, there is the advantage that it is possible to determine accurate gene numbers, if the cross is designed using *F. esculentum* as a tester. This was demonstrated in the study of winged seed. The character of winged seed was determined to be controlled by two genes. However, the inheritance pattern is that of a single gene in the intraspecific hybrids within *F. homotropicum* because the one of two loci was fixed as homozygous dominance. This fixed homozygous dominant locus was detected by more than one segregation pattern in the interspecific hybrids between *F. homotropicum* winged genotype and *F. esculentum* non-winged genotype. An erroneous conclusion would be reached if the study had included only intraspecific hybrids within *F. homotropicum*.

General Discussion of New Findings.

Interspecific hybrids between F. homotropicum and the two cultivated species. Interspecific hybrids between *F. homotropicum* 2x and *F. esculentum* have been developed by researchers (Campbell, 1995; Woo et al, 1999; Fesenko, I. N., 2001; Kim, S. K., et al.; Kim, Y. B., et al., 2001; and Zeller and Hsam, 2001). This thesis is the first report of the production of the interspecific hybrids from the crosses of *F. homotropicum* (4x) / *F. esculentum*, *F. homotropicum* (2x) / *F. tataricum*, and *F. homotropicum* (4x) / *F. tataricum*. These hybrids provide the access to the wild species *F. homotropicum* for improvement of the two cultivated buckwheat species. At the same time, *F. homotropicum* can be used as a bridge species to connect the two cultivated buckwheat species to overcome the difficulty of the direct hybridization. The data of crossability and hybrid recovery is useful information for phylogenetic studies. Interspecific hybrids between *F. homotropicum* and *F. tataricum* were obtained in this thesis while previous efforts to directly cross the two cultivated species was difficult with very limited success (Morris, 1951; Samimy, 1991; Hirose, et al., 1995; Chen, 1998; and Fesenko, I.N., et al, 2001). This indicates that *F. tataricum* might be more closely related to *F. homotropicum* than that to *F. esculentum*. However, further crosses between *F. homotropicum* and *F. tataricum* using more accessions are necessary to prove this hypothesis.

Study of the diploid and tetraploid forms of F. homotropicum. The previous study of the two forms of *F. homotropicum* was limited to isozyme analysis (Ohnishi and Asano, 1999). *In situ* hybridization was not successful in establishing the allotetraploid origin of *F. homotropicum* with 32 chromosomes because the two potential progenitors, *F. homotropicum* diploid and *F. esculentum* spp *ancestrale* were indistinguishable (Asano, 1998). Chromosome banding technique is not available due to the small chromosomes in *Fagopyrum* and the difficulty of chromosome preparation. No studies had been conducted on the crossability within each ploidy form and between two forms. The karyotype of two forms and chromosome pairing had not been examined and the karyotype between the two possible progenitors of *F. homotropicum* tetraploid had not been compared previously.

The two forms showed differences in karyotype and crosssability in this thesis. The karyotype analysis described 16 chromosomes of the tetraploid form as showing 8 extra chromosomes compared to the diploid form. The evidence of the allotetraploid origin of *F. homotropicum* tetraploid in this thesis was based on the similar karyotype of eight chromosomes between the tetraploid form and the diploid form and another eight chromosomes between the tetraploid form and *F. esculentum*. The bivalent chromosome pairing at meiosis and normal fertility were additional evidence of the allotetraploid origin. *Fagopyrum esculentum* had higher crossability with the tetraploid form of *F. homotropicum* compared to the diploid form, but *F. tataricum* had higher crossability with *F. homotropicum* diploid than with the tetraploid form. The diploid form showed unilateral crossability with *F. esculentum*, while the tetraploid form was bilaterally crossable with *F. esculentum*.

These results provide valuable information about the two forms of *F. homotropicum* and make it possible to efficiently utilize them in buckwheat improvement through interspecific hybridization. The study also helps to interpret speciation of *F. homotropicum* tetraploid. However, to confirm the allotetraploid origin of *F. homotropicum* tetraploid form, further studies are required, such as directly comparing karyotype of *F. homotropicum* tetraploid form with that of *F. esculentum* ssp. *ancestrale* and hybridization between *F. homotropicum* 2x and *F. esculentum* ssp. *ancestrale*.

Restoration of hybrid fertility and cytological variation of progeny in interspecific hybrid between F. homotropicum tetraploid and F. esculentum. This thesis reported an initial study in interspecific hybridization between *F. homotropicum* tetraploid and *F. esculentum*. Fertility restoration allows access to the *F. homotropicum* tetraploid in buckwheat improvement. The cytological analysis provides useful information for cytogenetic study. The aneuploid found in this study may be valuable genetic germplasm to locate genes on chromosomes and construct genetic linkage maps. In addition, spontaneous chromosome doubling and chromosome elimination in interspecific hybrids is the first observation in *Fagopyrum*.

Strategies to produce interspecific hybrids between the two cultivated species. The importance of interspecific hybridization between two cultivated species in buckwheat and the difficulty of this hybridization have been recognized. Attempts to hybridize the two species were made as early as the 1950's (Morris, 1951) and were repeated by several research groups (Samimy, 1991; Hirose, et al., 1995; Chen, 1998; and Fesenko, I.N., et al, 2001). Very limited success has been achieved. Techniques such as multiple hybridization and bridge cross have been applied in many important crops such as Brassicas, potatoes, and cotton to overcome the difficulty of crossing (Hadley and Openshaw, 1980; Vyas et al., 1995; and Vroh Bi et al. 1999), but these methods have never been used in buckwheat before. All previous attempts to cross the two cultivated buckwheat have used the direct cross.

The significance in success of hybridization between the two cultivated species in this thesis is not only in the production of interspecific hybrids themselves, but also in the demonstration of the strategies of interspecific hybridization in *Fagopyrum*. The significant improvement of hybrid viability in the bridge hybrids and the backcross between the bridge hybrid and self-pollinated buckwheat is promising because hybrid fertility might be fully restored in the future by repeatedly backcrossing. This possibility has been realized in other crops such as wheat (Ikeguchi, et al., 1999), cotton (Brubaker, et al., 1999), and sunflower (Bohorva and Atanassov, 1990). The hybrids from different types of crosses in this thesis had shown yield potential with increased growth vigor, branches, and clusters. It is expected that final fertile hybrid would combine the recombinant characters from the two cultivated species and wild species *F. homotropicum*. In addition, this study is the first report of unreduced gametes in interspecific hybridization in *Fagopyrum*.

Two gene model in inheritance of self-compatibility. Previous study in self-compatibility was mostly based on mutants of homostyly in *F. esculentum* (Sharma and Boyes, 1961; Marshall, 1969; Ruszkowski, M. 1980; and Fesenko, N. N. 1985 and 1986). It was suggested that self-compatibility could be controlled by either a supergene complex or multiple genes. However, self-compatibility in *F. homotropicum* might differ from

homostyle mutants in *F. esculentum*. One gene model with three allele series had been proposed previously based on the interspecific hybrids between self-compatible *F. homotropicum* and self-incompatible *F. esculentum* (Woo, et al., 1999). However, this model could not fully explain the segregation pattern in the BC_1F_1. The result of the same type of cross was interpreted by Fesenko, et al. (1998) as an indication that self-compatibility was not controlled by one gene.

The two gene model proposed in this thesis is based on analysis of F_2, F_3, BC_1F_1, and also of the pin x homostyly crosses. This is a new model in *Fagopyrum* as well as in plant kingdom for the inheritance of self-compatibility, which well explains the results from this study and previous studies. This study is useful for buckwheat improvement that involves in interspecific hybridization between the self-compatible and self-incompatible species. The first gene of self-compatibility has been identified by RAPD markers and a co-dominant SCAR marker has been developed (Aii et al., 1999). Base on this marker and the present study, the second gene may be detected by molecular technique in the future.

Inheritance of seed shattering based on interspecific hybrids between F. esculentum and F. homotropicum. It was established that one gene controls seed shattering in *F. esculentum* spp *ancestrale* (Ohnishi, 1999), but the inheritance of seed shattering in *F. homotropicum* was not clear. The 3:1 ratio in the F_2 (one gene) reported in the interspecific hybrid between *F. homotropicum* and *F. esculentum* has not been confirmed (Campbell, 1995 and Wang and Campbell, 1998). Based on three segregation patterns 3:1, 9:7, and 27:37 in separate F_2 populations, this thesis proposed a three gene model. The different segregation ratios in the F_2 reflected the heterogeneity of *F. esculentum* due to cross-pollinating. This model is the first study to explain the inheritance of seed shattering in the different segregation patterns. Seed shattering caused severe yield loss. Cultivated buckwheat is seed non-shattering, however, seed shattering become a considerable problem when shattering species of *F. homotropicum* is used for buckwheat improvement. The three gene model provides a guidance to breed non-shattering buckwheat. It was noticed that shattering could be produced from the cross of two non-

shattering parents and on the other hand, non-shattering could be produced from a heterozygous shattering plant.

Possible further studies include linking the three genes with physiological markers, for example, abscission layers on pedicels to allow ease of selection, and to detect molecular markers for application of molecular marker assisted selection.

Inheritance of winged seed based on intraspecific and interspecific hybrids. Very little information is available on the inheritance of winged seed in *Fagopyrum* and other plants. Wang and Campbell (1998) could not determine the inheritance pattern based on a bulked F_2 population in interspecific hybrids between *F. homotropicum* and *F. esculentum*. The 9:7 ratio in the F_2 population reported by Zeller and Hsam (2001), suggested that two genes might control the character. However, inheritance studies based on a single F_2 population or a bulked population could not fully represent all of the segregation patterns in interspecific hybrids due to the heterogeneity of *F. esculentum* from cross-pollinating. In this thesis, a two gene model to explain genotypes of *F. homotropicum* and *F. esculentum* was proposed based on individual F_2 populations that were derived from F_1 single plants in interspecific hybrids. Inheritance patterns in interspecific hybrids were compared with those from intraspecific hybrids from *F. homotropicum*.

This is the first inheritance study on the winged seed character with experimental design in both interspecific and intraspecific hybrids. It is the first report in *Fagopyrum* that identified the difference in inheritance patterns between interspecific hybrids and intraspecific hybrids. This study provides useful information of winged seed inheritance in buckwheat, and also contributes the understanding of the character in other plants. Cultivated buckwheat has non-winged seed. Winged seed was introduced into interspecific hybrids when wild species *F. homotropicum* was used to improve common buckwheat. Winged seed reduces seed density and causes problems for the cleaning equipments. This study will facilitate efficient selection of non-winged seeds in the breeding program.

Conclusions.

1. Interspecific hybrids between *F. homotropicum* and the two cultivated species can be produced assisted by ovule rescue technique.
2. *Fagopyrum homotropicum* tetraploid is similar to *F. homotropicum* diploid in fertility but distinct in karyotype and crossability. This thesis provides more evidence of allotetraploid origin of *F. homotropicum* tetraploid.
3. The fertility of interspecific hybrids between *F. homotropicum* tetraploid and *F. esculentum* can be restored by colchicine treatment. The resulted progeny were hexaploid after chromosome doubling, diploid after chromosome elimination, and aneuploid from both chromosome duplication and elimination.
4. Interspecific hybrids between the two cultivated species can be produced through direct, multiple, and bridge cross. However, the hybrids from bridge cross using *F. homotropicum* as a bridge species had higher pollen viability than the other of crosses.
5. Self-compatibility is controlled by two complementary genes with a three alleles S, S^h, and s at the first locus, and two alleles, S_c and s_c at the second locus.
6. Three genes control seed shattering with shattering dominant to non-shattering. Seed shattering could be produced from crosses between the two non-shattering parents, while non-shattering could be produced from crosses between the two shattering parents or from selfed shattering plants.
7. Winged seed was dominant to non-winged seed and was controlled by two genes Inheritance in intraspecific hybrids within *F. homotropicum* acted as a single gene inheritance due to fixed homozygous alleles at both loci, while inheritance in interspecific hybrids between *F. homotropicum* and *F. esculentum* shown both one gene and two gene patterns due to heterogeneity of *F. esculentum* from cross-pollinating.

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6.0 APPENDICES

6.1 Ovule Rescue Technique in Interspecific Hybridization

Pollination. The pin flowers (self-incompatible) of common buckwheat used for crossing were emasculated in the very early morning before flower opening or without emasculation. The self-compatible flowers of *F. homotropicum* and *F. tataricum* were emasculated in the afternoon the day before pollination. The pollination was carried out in the morning before 11:00 o'clock. Both emasculation and pollination were done by means of a hand magnifier and flowers were bagged for 3-4 days after pollination.

Ovule rescue. After pollination, these enlarged ovaries (seeds) were rescued at 5-19 days, most at 7-10 days before abortion. The ovaries were surface sterilized in 70% ethanol for 30 seconds and in 10% commercial bleach for 50 seconds and then followed by 5 rinses in sterilized distilled water. The ovules were carefully excised from the ovaries and plated in the test tubes (25 x 100 mm) (1 ovule per tube), which contained 10 ml medium. The full or half strength MS medium (Murashige and Skoog, 1962) with or without modification (Appendix 6.2) was used in various crosses.

Culture conditions and plant regeneration. Ovule cultures were kept at 22 °C with continuous light at the intensity of 40 μmol m^{-2} s^{-1}. The embryos emerged from ovules in approximately 1-2 weeks. The embryos often developed into the plantlets in the half strength MS medium without further medium transfer. The embryos that derived from other culture media often developed into callus or abnormal tissue that required to go shoot and root induction. Shoot induction used MS modified medium as described by Samimy et al. (1996), containing the MS salt mixture and vitamins, 3% sucrose, 2 g l^{-1} casein hydrolysate, 0.2 mg l^{-1} IAA (indoleacetic acid), 2.0 mg l^{-1} zeatin and 0.25% Phytagel. Shoots 1-2 cm in length were transferred to a rooting medium. The media for rooting was either half strength MS or half strength MS supplement with 3 mg l^{-1} IBA (indolebutyric acid) or 10 mg l^{-1} IAA. The rooting was slower in the first than the latter media. Rooting by vermiculite was another method, with Stim-Root No.2 (0.4% rooting power IBA) (Plant Products Co. LTD. Rrampton Ontario Canada L6T 1G1) applied to

the bottom of the shoot before it was inserted into the sterilized fine vermiculite that was soaked by tap water in a magenta box (65 x 65 x 100 mm). This method produced good root development and the plantlets had good survival rate following transplanting in the greenhouse. Plantlets with fully developing leaves and roots in the magenta boxes were directly transplanted into the greenhouse. Those plantlets that rooted in the medium were first transplanted into the plastic micropots (70 x 50 x 50 mm) containing mixture of sterilized soil and vermiculite (1:1), covered with a clear plastic lid for one week and then into the greenhouse.

6.2 Media Used for Ovule Culture in Interspecific Hybridization

Medium Name	Medium Composition (one liter)	Crosses
1/2 MS	half-strength MS	E x H, T x H
1/2 MAZ	2/1MS,10mg L-tyrosine,10mg L-arginine,2mg zeatin	E x H
MAZ	MS, 10mg L-tyrosine, 10mg L-arginine, 2mg zeatin	T x H
MZ	MS, 2mg zeatin	T x E
MZC	MS, 2mg zeatine, 2g casein hydrolysate, 100mg inositol	T x H

E: *F. esculentum*; T: *F. tataricum*; H: *F. homotropicum*.

All media contained 3% sucrose and were solidified by 0.7% agar.