Isolation, description, inheritance, associated traits and possible uses of three barley (Hordeum vulgare L.) starch mutants
by Tae Young Chung

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Abstract:
Two barley pericarp starch mutants that retained starch in the pericarp layer at maturity and one barley endosperm mutant, designated "fractured" starch in which the endosperm consisted of angular, and what appeared to be fractured starch granules, were identified, the inheritance determined, and the characters associated with these mutants , and uses related to the human consumption determined. All the mutants were inherited as single recessive genes and the fractured starch mutant expressed xenia. The symbols per 1and per 2 for the pericarp starch mutant 1 (Pernubet I) and 2 (Pernubet 2) genes and fra for fractured starch gene (Franubet) were assigned. The linkage relationship between starch mutants and translocation breakpoints were determined using the translocation homozygote lethal stocks and the per 1, per 2 and fra genes were located in chromosomes 1, 7 and 4, respectively. Small seed size was in common for all three mutants. Fewer kernels per spike, high tillering ability, high lysine content and low ß-glucan viscosity were associated with the fractured starch mutant. Most of the viscous substances appeared to be synthesized near physiological maturity of the kernel. The waxy endosperm genotype had significantly higher ß-glucan viscosity and fractured starch mutant had significantly lower than their parent Nubet isotype. The flour yield of Franubet was much greater than that of Nubet and this appeared to be associated with lower ß-glucan viscosity. The kernels of waxy endosperm and hulless genotypes pearled slower than that of their parent isolines, though the pearling indexes were effected by the sample size charged, moisture content of the kernel and pearling times. For the comparisons of genotype differences at the optimum pearling index a method of adjusting pearling index with the ash content was developed. No pronounced differences were observed between Nubet and starch mutant diets on the gain per day, feed consumption, or digestible energy in a rat feeding trial and energy balance study.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Two barley pericarp starch mutants that retained starch in the pericarp layer at maturity and one barley endosperm mutant, designated "fractured" starch in which the endosperm consisted of angular, and what appeared to be fractured starch granules, were identified, the inheritance determined, and the characters associated with these mutants and uses related to the human consumption determined. All the mutants were inherited as single recessive genes and the fractured starch mutant expressed xenia. The symbols *per 1* and *per 2* for the pericarp starch mutant 1 (Pernubet 1) and 2 (Pernubet 2) genes and *fra* for fractured starch gene (Franubet) were assigned. The linkage relationship between starch mutants and translocation breakpoints were determined using the translocation homozygote lethal stocks and the *per 1*, *per 2* and *fra* genes were located in chromosomes 1, 7 and 4, respectively.

Small seed size was in common for all three mutants. Fewer kernels per spike, high tillering ability, high lysine content and low β-glucan viscosity were associated with the fractured starch mutant. Most of the viscous substances appeared to be synthesized near physiological maturity of the kernel. The waxy endosperm genotype had significantly higher β-glucan viscosity and fractured starch mutant had significantly lower than their parent Nubet isotype.

The flour yield of Franubet was much greater than that of Nubet and this appeared to be associated with lower β-glucan viscosity.

The kernels of waxy endosperm and hulless genotypes pearled slower than that of their parent isolines, though the pearling indexes were effected by the sample size charged, moisture content of the kernel and pearling times. For the comparisons of genotype differences at the optimum pearling index a method of adjusting pearling index with the ash content was developed.

No pronounced differences were observed between Nubet and starch mutant diets on the gain per day, feed consumption, or digestible energy in a rat feeding trial and energy balance study.

Chapter 1

INTRODUCTION

Alternative uses of barley include industrial utilization, food, feed and malting. Researchers at Montana State University are working to improve the cash return from barley production. The objectives of this project are to increase the usage of barley and barley products for human consumption with quality acceptable to the consumer.

Korea has traditionally used barley as a food, but the consumption of barley has been declining drastically. This reduction in barley consumption is due to recent substantial gains in rice production and consumption. The reason for this replacement in Korean diets is that the rice grain is considered to be more palatable, attractive, and convenient than pearled barley.

The purpose of this study was an attempt to find a barley mutant which would be more like rice. The characters related to the industrial, food and feed uses, and inheritance of new induced barley mutants were investigated.

Although this study did not develop a barley with acceptable cooking quality for the Korean consumer these results may contribute to finding a barley more nearly like rice.

Chapter 2

LITERATURE REVIEW

Chemical Mutagenesis

Induced mutations are a suitable breeding method when new or specific breeding objectives are to be realized. It is a method which enlarges the genetic variability of a crop and may produce new, suitable forms necessary for attaining distinct breeding objectives. It has become one of several valuable tools in varietal improvement of barley and is becoming increasingly important (Scholz, 1971). Its importance is demonstrated, especially in barley, by the release of new, improved commercial varieties. According to Sigurbjoernsson (1975), 36 barley varieties were released between 1962 and 1975. All these varieties were by direct mutation or from crossing a mutant with other cultivars or lines.

A number of chemical mutagens are available to induce point mutations. Of these agents only a few are really useful for inducing mutations in cultivated plants, and most of these are alkylating agents. Nilan (1964) stated that diethyl sulfate was one of the most important mutagens for barley. With appropriate treatments, diethyl sulfate induced negligible frequencies of chromosome aberration, very little physiological injury and a higher proportion of viridis than albino chlorophyll seedling mutants. They suggested that mutagen concentration, treatment duration, pH, temperature during treatment, oxygen supply, seed-size and caryopsis type are the important factors influencing the deleterious side effects and mutation rates. Nilan also reported that type of treatment is more important than concentration in reducing deleterious side effects and increasing the mutagenic effectiveness. Soaking barley in 0.01M diethyl sulfate solution for 6 hours was recommended.

Nilan et al. (1963) also suggested a buffered solution at pH 7.0, a temperature of 10 C during treatment, and adequate oxygen supply to induce higher mutation frequencies with less physiological injury. Small seeds and hulless seed treated for the same length of time as large seeds or hulled seeds showed more physiological damage and significantly higher mutation frequencies.

Heiner (1963) found that the ethyl group of diethyl sulfate preferentially reacted with guanine of DNA to induce mutations in barley.

Translocation

Since barley translocations were found by Smith (1941), they have been used as a tool for special cytological studies and offer enormous potential for plant breeders to engage in chromosome engineering (Ramage, 1963).

Burnham (1962) described a method using translocations to test for the independence of barley linkage groups. Kramer et al. (1954), using translocation-gene linkages and F_1 meiotic configurations of translocation intercrosses, concluded that two linkage groups (linkage groups III and VII) were carried by the same chromosome (chromosome 1). The Fourth American Barley Research Workers Conference adopted a system of designating the chromosomes and the linkage groups. In this system, Arabic numbers 1 through 7 are used to designate chromosomes and linkage groups. Extensive genetic data have been compiled using this system and published at regular intervals (Nilan, 1964; Robertson, 1967; Robertson, 1971).

Barley translocations have been used by a number of workers to assign new mutants to specific chromosomes and to test linkages. Homozygous translocations have little or no

effect on the phenotype of barley though they may alter linkage groups and chromosome morphology (Burnham, 1962). Heterozygous translocations are phenotypically recognized by semisterility as a result of aborted spores. The aborted spores are caused by deficiencies and duplications of a chromosome segment resulting from adjacent disjunction (Ramage, 1963).

Pollen and ovule abortion were higher than 50% in heterzygous translocations of corn (Burnham, 1962). Barley translocations, however, averaged about 25% in the ovule and 29% in pollen. Abortion was mainly due to an excess of alternate disjunctions. When crossing-over in the interstitial segments is followed by alternate disjunction, these crossed-over chromatids in spores are deficient in genetic information and abort. An important consequence of this excess alternate disjunction in barley is that very few recombinants are recovered from genes occurring between the centromeres and breakpoints of translocations (Ramage, 1963).

Hanson (1952), as cited by Burnham (1962), reported the pattern of crossing-over reduction in the interstitial segment using linkage data obtained with three known markers (K, Lg_3, bl) in barley chromosome 4 and three translocations (T2-4a, T3-4a, T4-5a) involving this chromosome.

Ramage (1966) also reported that 13 translocations involving chromosome 2 were tested against the male sterility gene, *msg 2*. The range of recombination values were 0 to 2 units in the interstitial segment. Therefore, the pattern of crossing-over reduction in the interstitial segment furnished the position of the centromere and the linkage data provided the distance between the centromere and markers (*msg 2*).

When an individual heterozygote for a translocation and for a gene pair is selfed, four phenotypic classes can be recognized in the F_2 generation. These classes are, semisterile dominant and recessive, and fertile dominant and recessive. Either of the semisterile classes on fertile classes provides two measurements of linkage information. The semisterile-recessive zygotes arise from the union of a recombinant and a nonrecombinant gamete. Normal-recessive and homozygous translocation recessive zygotes arise from the union of two nonrecombinant or two recombinant gametes. Therefore, the recessive classes give more information about linkage per individual than the dominant classes (Tuleen, 1971). If homozygous translocation recessive and normal recessive classes can be determined, the amount of recombination information will be maximized. Root-tip squashes or a test cross method were suggested to determine the homozygous translocations (Burnham, 1962; Tuleen, 1971).

Since a translocation involves an exchange of pieces between two non-homologous chromosomes, the linkage obtained is evidence for the location of assigned gene actions in either or both chromosomes. Therefore, linkage information may provide 'pseudo-linkage' between genes and one of the chromosomes involved in the translocation. To determine which of these possibilities is true, a test must be made with an additional translocation involving a break-point at nearly the same locus in one of the chromosomes or the other breakpoint in this additional translocation being in a different chromosome (Burnham and Cartledge, 1939).

All possible combinations (21) of translocated chromosomes have been isolated. The recommended designations of translocations along with the break-position, and recombi-

nation values of genes and breakpoints in heterozygous translocations have been summarized (Ramage et al., 1961; Ramage and Suneson, 1961; Hagberg et al., 1978).

A translocation tester set involving seven barley chromosomes has been used extensively to identify the various gene locations and as a source of marker genes.

Genes conditioning shrunken endosperm (Jarvi and Eslick, 1975; Ullrich and Eslick, 1978), male sterile (Eslick et al., 1972), seedling lethal (Rahman and Eslick, 1975), erectoid (Bockelman and Eslick, 1977), scald resistance (Bockelman et al., 1978) and high amylose gene (Ullrich and Eslick, 1978) have been identified along with associated linkage groups. The balanced male sterile-translocation system with dominant preflowering selective genes was proposed to produce the female stocks in commercial hybrid seed production. Eslick (1971) suggested that translocations created the necessary linkages which is one of the problems in previous hybrid systems.

The utilization of translocation homozygote lethals were initially proposed by Eslick (1972) as a useful breeding tool. The possible advantages of these translocation homozygote lethals are (1) maintaining recessive male steriles and albino genes or other lethals in a heterozygous stock without roguing homozygous dominant plants, (2) developing balanced male sterile stocks, (3) increasing precision of linkage studies and eliminating the root-tip squash method or test cross for identifying homozygous translocations, (4) transferring desirable genes to a recurrent parent without checking characteristics which may require expensive or tedious laboratory work (Biggerstaff and Eslick, 1978).

Kernel Structure and Mutants

The barley grain of covered cultivars consists of hull, pericarp, integuments, endosperm and embryo. As the barley ripens the hull and pericarp becomes firmly attached to

the wall of the kernel. The structure and adherence of hull are important characteristics for germinating grain in the malting process. The adhering hull protects the seedling from mechanical damage, and restricts excessive seedling growth without affecting the desirable enzymic degradation (Pomeranz and Bechtel, 1978).

According to Reid and Wiebe (1979), naked caryopsis (hulless kernels) are frequently found as a result of natural mutation, especially in primitive mountainous areas where barley is used for human food. Nilan (1964), in his genetic review of barley, noted that naked caryopsis *n* is controlled by a single recessive gene. It is located on the long arm of chromosome 1.

Eslick et al. (1972), from the three point linkage tests, indicated that the *n* gene is located between lk_2 (short-awn) and *msg 10* (male-sterile) and the recombination values between *msg 10* and *n*, *n* and lk_2, and *msg 10* and lk_2 were 7.2, 7.9, and 14.7 units, respectively. Since translocation data indicated that the *msg 10* locus is located very near the centromere region, the *n* gene is probably located 7.2 units from the centromere in the long arm of chromosome 1.

Isogenic analysis of the covered and naked caryopsis isotypes developed with different varietal backgrounds revealed that the average yield reduction in a wide range of environments by naked caryopsis was 12%. It was concluded that the kernel weight and yield reductions of naked barley are probably proportional to the weight of the hulls (8-16% of the kernel weight) (Eslick, 1979). Since the hulls of naked barley are easily separated from the caryopsis during the threshing process, the proportion of the seed components changes accordingly. The protein, starch, and fat concentration of the seed increase due to the reduction in the crude fiber represented by the hulls (Newman et al., 1968).

It was suggested that the advantages of hulless barley to the end user are (1) 30% less storage space, (2) superior by-products from processing, (3) reduced energy requirements for syrup or starch production, (4) 12% less weight to transport, and (5) for the plant breeder, rapid visual selection for potentially important biochemical mutants (Eslick, 1979).

The pericarp consists of the epidermis, hypodermis, cross cells, and tube cells. The remaining tissues of the grain are seed coat nucellar tissue and endosperm (Pomeranz and Bechtel, 1978).

During kernel maturation, large numbers of small, spherical starch granules were detected in the pericarp of barley kernels shortly after anthesis. These granules were very quickly digested by the alpha-amylase present in the pericarp and 15 days after anthesis no pericarp starch remained in the kernels. It was suggested that the function of the alpha-amylase is to hydrolyse the pericarp starch to provide energy for the growing kernel (MacGregor et al., 1972).

To facilitate a study of various tissues in small grains a staining procedure was developed for use in conjunction with a Strong-Scott barley pearler (Scheuring and Rooney, 1979). May-Grunwald solution (0.5 methylene blue and 0.5% eosin-Y in methanol) stained the germ, pericarp, and starchy endosperm of sorghum blue, green, and pink, respectively. The method was suggested for use in studying pearled grain.

The aleurone layer consists of large rectangular, heavy walled starch free cells. Botanically the aleurone is the outer layer of the endosperm (Pomeranz and Bechtel, 1978). It has been suggested that the aleurone layer is a specialized secreting tissue situated at the periphery of the starch endosperm in seeds. During germination, extensive degradation of

the aleurone cell wall under the action of gibberellic acid stimulation supports the enzyme releasing function (Taiz and Jones, 1970). The proportion of aleurone was estimated to be 6.9% of whole grain in covered barley and to contain 21.5% of the protein (Novacek et al., 1966).

Endosperm Starch and Mutants

Starch consists of two distinct molecular forms of polymerized glucose molecules, amylose; a linear homopolysaccharide, and amylopectin; a branched homopolysaccharide. Amylose is composed of alpha-D-glucose units with alpha-1, 4 linkage and the amylopectin is composed of alpha-D-glucose units linked in straight chains by alpha-1, 4 with branch points being alpha-1, 6 linkages. A typical cereal starch consists of 75% amylopectin and 25% amylose (Banks and Greenwood, 1975).

Goering et al. (1957) found that the amylose content of starch in 30 samples of Compana barley range from 19 to 25% on a dry basis. In 44 different varieties of barley, amylose content varied from 13 to 24% of the total starch, illustrating that inherent differences exist in amylose to amylopectin ratios.

Scanning electron microscopy has revealed principally two sizes large round to oval types over 25μm in size and small starch granules about 5 μm, both embedded in a matrix of reserve protein in barley endosperm (Pomeranz, 1974).

Since the cereal starch granule is formed within the plastid which controls shape and structure, most cereal starch granules are single spherulitic structures. However, one plastid may give rise to a multiplicity of nuclei, and subsequently compound granules are formed (Banks and Greenwood, 1975). The starch granules of rice and wrinkled-seeded peas are

compound and fairly angular as illustrated by electron microscopic photographs. Those starch granules are complex and exhibit a central cavity (Banks and Greenwood, 1975; Juliano et al., 1975).

Williams and Duffus (1977) showed the development of the amyloplast in the barley endosperm. At two days after anthesis amyloplasts could be seen in the endosperm, each containing many small starch granules. After this period, some enlarge to become full-sized large granules. By 14 days after anthesis two populations of amyloplast, large and small, appeared, the large being about 11 μm across and the smaller 3 or 4 μm across.

A wide varietal range in the ratio of small to large granules from a minimum of 5.5:1 to a maximum of 37:1 on a number basis were reported. Small granules accounted for 6.2 to 30.6% of the total starch weight (Goering et al., 1973b). No substantial differences were found between large and small starch granules separated from mature barley for % protein, % fat, swelling power, iodine affinity and Brabender cooking viscosity curves indicating that small granules observed in mature barley endosperm are a second discrete population of starch granules and not immature granules (Goering and DeHaas, 1974).

The texture of cooked rice is determined largely by the amylose/amylopectin ratio of the starch (Juliano et al., 1964). According to Juliano (1979), in a review on rice quality, two attributes of cooked rice commonly measured are tenderness (softness) and cohesiveness (stickiness). These are inversely related with amylose content, especially hot water insoluble amylose content. Amylose content of milled rice varied from 9 to 33% among the selected samples (550 samples collected from 18 counties). Japonica types, which are acceptable in China, Korea and Japan, showed low to medium amylose content (9-15, 15-20%, respectively).

Waxy barley endosperm contains very little (0-3%) amylose and almost 100% amylopectin in the endosperm starches (Goering et al., 1973a). This character can be identified by an iodine reaction on the endosperm and pollen starch grains. Iodine reacts with normal starch producing a deep blue color while the reaction with the waxy starch produces a reddish-brown color (Haus, 1975).

Nilan (1964) summarized the genetic studies on the waxy endosperm character. The waxy endosperm gene, *wx*, is a simply inherited recessive, and normal allele is completely dominant in *Wx wx wx* and *Wx Wx wx* endosperm and expresses xenia for the trait. This gene belongs to the chromosome 1 linkage group and is located on the short arm approximately 50 recombination units from the centromere. The waxy gene produces no noticeable gross differences in crop appearance except that it imparts an opaque hue to the grain in contrast to the more vitreous appearance of normal grain (Eriksson, 1969).

The waxy endosperm gene was introduced into established barley cultivars because of the observation that waxy starch is more readily modified by enzymes and chemicals than normal starch (Goering et al., 1973b). Low pasting temperature and easy hydrolysis of waxy barley allows complete conversion without conventional cooking temperatures and times. By using waxy barley, high maltose syrups (maltose contents were 58 to 66%) were produced in the laboratory and pilot plant (Goering et al., 1980). Waxy endosperm has also been reported in rice, corn, and sorghum. Amylose content and starch properties of waxy rice, corn, and sorghum are similar to the waxy barley (Medcalf, 1973). Waxy rice is high in cohesiveness and very sticky because of high amylopectin content (Juliano et al., 1965).

High amylose endosperm was reported in Glacier barley by Merritt (1967). The amylose content in the endosperm of this mutant was 44% compared to 24% in the original parent. The mutant has been designated as *ac 38* and was inherited as a simple recessive. This mutant gene exhibited a dosage effect and also expressed xenia. The gene was assigned to chromosome 2 as determined by the trisomic analysis method in Betzes background (Ullrich and Eslick, 1978). Scanning electron micrographs revealed that high amylose starch granules of barley formed irregular shapes and were significantly smaller than normal starch granules (Banks and Greenwood, 1975). Two high amylose genes (amylose content 60-70% of starch) were also reported in corn. The starch developed in high amylose corn has peculiarly shaped granules. Long bulbous granules were found clumped together and surrounded by other types of granules. High amylose starch has an extremely high gelatinization temperature and is exceedingly resistant to the action of digestive enzymes (Sandstedt, 1965).

Because of the linear nature of amylose, the high amylose grains are of commercial interest. The amylose has a number of potential uses which a branched polymer does not. Linear polymers are particularly suited for film and coating applications. Edible films which are only slightly permeable to gases could be used as containers for foodstuffs and present no waste disposal problems (Medcalf, 1973).

Reid and Wiebe (1979) referred to a kernel type in barley in which the starch was replaced by a sugary liquid. As the seed matured it collapsed and the collapsed seed failed to germinate. Stocks of this mutant could be maintained as heterozygotes which expressed xenia.

Sugary endosperm genes (*su 1* and *su 2*) high in sucrose were also reported in corn. These sugary endosperms stored less starch. Instead, they contained four to ten fold more sugar than normal endosperm types. The additive effects in sucrose content of *su 1 su 2* genotype were exceedingly small and were largely aggregated into compound granules which are somewhat analogous to wrinkled pea starch (Sandstedt, 1965).

Gene interactions of waxy, high amylose, and sugary endosperm have been studied for starch properties and structure in corn. The high amylose gene (*ae*) and the waxy (*wx*) were shown to be completely epistatic to high amylose (*du*) and sugary (*su 1, su 2*) in amylose content. The *ae wx* genotype had 15% amylose in the endosperm starch (Kramer et al., 1956).

The complexity of the starch granules is increased under the influence of two or more homozygous recessive genes. The combination of high amylose (*ae*) and the sugary (*su 1*) genes produces a mixture of many kinds of granules. Some were made up of many exceedingly small blue staining particles (some of which were birefringent) embedded in a red staining and nonbirefringent matrix (Sandstedt, 1965).

Protein Synthesis and Mutants

As a percentage of the tissue, protein is highest in the embryo, next highest in the bran, and lowest in the endosperm. Protein stored in the endosperm is inversely related to carbohydrate content. High protein content in grain is therefore at the expense of stored starch (Canvin, 1976).

Sixty-five generations of continuous selection of corn for high and low crude protein extended the upper level to 25% and the lower level to 4%. Yield capacity and seed size, however, were depressed in high protein selections (Dudley and Lambert, 1969).

Johnson et al. (1969) suggested that a single genotype of wheat could produce grain varying from as low as 8% protein to as high as 18% depending on the environment in which it is grown. The environment affects protein content of grain to a greater extent than the genetic system and these two effects are difficult to separate (Johnson et al., 1969).

Barley cultivars responded differently in grain protein content to increasing levels of nitrogen application. Malting barley cultivars showed a remarkable increase in protein content, however, very little change occurred in Hiproly and C.I. 4362 (McGuire et al., 1979).

The endosperm protein consists of high amounts of glutamic acid and proline while glycine, valine, and arginine are much lower in the endosperm compared to embryo and aleurone protein. The protein in the germ and aleurone cells contain considerably more of the essential amino acids, arginine, histidine, lysine, methionine, threonine, and valine (Munck, 1972).

Since the opaque-2 endosperms in corn which had a different amino acid pattern and 69% more lysine than the normal corn, intensive investigations have been done to find high lysine mutants in various crops (Mertz, 1976).

The high-lysine barley, Hiproly, of Ethiopian origin, isolated from the world barley collection (Munck et al., 1970) and the mutant Risø 1508, produced by chemical mutation (Ingverson, 1975), contain significantly higher lysine (Hiproly, 30% and Risø 1508, 45%) than their parents. The mode of inheritance of the high lysine trait of Hiproly and Risø 1508 were determined to be a single recessive (Munck et al., 1970; Doll, 1973).

From the study of classical Osborne protein fractions in these mutants during the grain filling period, it was found that high lysine in Risø 1508 derived from Bomi is due to the depression of hordein synthesis. The albumin fraction in normal barley is synthesized early in grain development, whereas hordein, glutelin and globulin are synthesized from 10 days after fertilization until maturity. The high lysine mutants, on the other hand, showed a remarkable depression of hordein and globulin synthesis with increasing albumin (Cameron-Mills et al., 1979).

Jarvi and Eslick (1975) and Ullrich (1978) identified six and eight shrunken endosperm lines and found that the mutants contained high lysine. The high lysine traits are controlled by single recessive genes and some of them expressed xenia. All of the mutants including Hiproly and Risø 1508 showed lower grain yield and test weight because of shrunken endosperms. Linkage or pleiotropy between high lysine and shrunken endosperm are assumed because the association appears to be quite general in barley.

β-Glucan Viscosity and Brabender Viscosity

Gums extracted from raw barley and malt with warm water have presented problems in the brewing industry by causing increased viscosity and filtration problems in the sweet wort. In the finished beer the remaining high molecular weight substances originating from barley gums influence quality. They raise the viscosity and improve the foam stability of beer. Gums from barley, malt, and wort were hydrolysized to glucose, arabinose, and xylose with acid (Djurtoff, 1958). Scott (1972) found that most of the specific viscosity of worts was contributed by β-glucan. The viscosity of the worts due to other components remained constant among varieties.

β-Glucan is a general name for all non-cellulose compounds of two or more glucose molecules linked together in the β-configuration (Bourne and Pierce, 1972). The barley β-glucans are essentially linear molecules containing both β-1, 4 and β-1, 3 glucosidic linkage which are randomly arranged (Preece and MacKenzie, 1952; Igarashi and Sakurai, 1965).

Most methods of β-glucan extraction methods are based on precipitation from aqueous extracts (Bourne and Pierce, 1972). If only the hot water soluble gums are extracted, the β-glucan estimates tend to be low. Estimates increase if both water soluble and insoluble fraction are extracted using strong acid or basic solutions (Forrest and Wainwright, 1977).

For breeding purposes, a rapid viscosity method for estimating β-glucan has been developed (Greenberg and Whitmore, 1974; Morgan and Gothard, 1977; Morgan, 1977). The amount of β-glucan is not measured directly, but is estimated by the amount of viscosity produced by an extract. Greenberg (1974) found that extract viscosity was closely related to actual β-glucan content in a logarithmic fashion, with a correlation coefficient of 0.89.

β-Glucan is believed to be situated mostly in the endosperm as a part of the cell walls and material surrounding the starch granules (Bourne and Pierce, 1972). It was suggested that the β-glucans were contained in the barley aleurone cell walls (Taiz and Jones, 1970), however, McNeil and Albersheim (1975) found that the aleurone cell walls are composed of arabinoxylan and cellulose. Fulcher et al. (1977) suggested that the main β-glucan deposition is at the sub-aleurone cell walls that are immediately adjacent to the aleurone layer. Fluorescent microscopic photographs illustrated considerably more sub-aleurone cell wall than the remainder of the endosperm and these sub-aleurone cell

walls contained extensive deposition of aniline-blue positive material indicating β-glucan deposition (Fulcher et al., 1977; Wood and Fulcher, 1978). Gohl et al. (1977) showed the distribution of the viscosity within the matured barley kernel using pearled fractions. The layer between the bran and the center of the kernel revealed the highest viscosity.

The pentosan and hemicellulose content also influence viscosity (Bourne and Pierce, 1972). The materials extracted by a 4% aqueous sodium hydroxide solution from cereal grains after removal of starch and gum, are hemicelluloses. Two types of hemicellulose, a husk type and an endosperm type were obtained. The husk type was found to have a low viscosity and the endosperm type was high in viscosity.

Current thinking is that gums and hemicellulose are all derived from the same basic source and pure extraction is difficult (Forrest and Wainwright, 1977).

Varietal differences in β-glucan concentration was reviewed by Bourne and Pierce (1972). Hot and dry growing conditions increase the glucan concentration and wet growing conditions appear to favor low viscosity. Varietal differences, however, remain in the same order.

In an isogenic study on barley viscosity, using caryopsis type, waxy endosperm and short awn genes and their normal counterparts, Fox (1981) detected a significant difference in extract viscosity among genotypes. Naked, short awn and waxy recessive genotypes were higher in viscosity than their parents. Three recessive double recombination types were higher in viscosity indicating additive gene action among those genotypes for β-glucan viscosity of the grain.

Intensive studies on the Brabender cooking viscosities have been done with barley flours and starches to evaluate the starch and flour characteristics. Goering et al. (1970)

observed some genetic differences for cooking viscosity among starches from 12 barley genotypes. In general, the hulless varieties have a higher paste viscosity than the covered types. Some varietal differences were also determined. Starch from Nupana showed the highest viscosity and Titan the lowest. Paste gelatinization temperatures were similar among non-waxy cultivars.

Starch from the waxy genotypes has been compared to starch from normal genotypes (Goering et al., 1973a). Waxy starches showed higher pasting peak at about a 20 C lower temperature, had more granule instability and very little setback on cooling compared to their normal starch counterparts. Small and large starch granules isolated from 3 different barley isogenics including high amylose, waxy and high lysine isotypes with their derived lines were investigated for starch properties (Goering et al., 1975). No significant difference of the Brabender cooking curves between small and large starch granules was found. These results indicated a similar stability for small starch granules compared to large granules during cooking.

Cooking viscosity curves of barley flours were similar to the starch curves for genotype differences. Maximum viscosities, however, are relatively lower than starch viscosities (Goering et al., 1980).

Brabender amylographs have been used to determine gelatinization temperature on milled rice flour and rice starch and as a means to index eating quality of rice. Correlation studies indicated that peak viscosity and setback characteristics on the amylogram were related to palatability, stickiness and amylose content among the Japonica and Indica types. The preferred rice in Japan has high peak viscosity, low temperature to attain peak viscosity and greater breakdown (setback). The variation of gelatinization temperatures of

the acceptable rice varieties has been shown to be from 55 C-79 C with 65-68 C as the preferred range (Suzuki, 1979).

Water Absorption of Starch Mutants

According to Pratt (1964), water uptake by wheat flour is influenced by the protein and starch content in the flour with only minor influences caused by the other constituents, such as dextrins, pentosans and cellulose. Sorum (1977) reported the alkaline water retention capacities (AWRC) of barley flour. High water retention capacity was found in barley flours compared to wheat flours.

The swelling properties of starch are due to the contribution of the hydroxylated nature of the D-glucose molecules, the large molecular size of the constituent polymers, and the granular form itself (Medcalf, 1973). Swelling power of waxy starch granules extracted from the waxy isogenic pairs of Compana and Oderbrucker were compared to the normal starch granules. Waxy starch showed higher swelling power than starch from the normal isotypes (Goering et al., 1973a).

An excellent review on the water absorption of barley grain was reported by Brookes et al. (1976). Positive correlation between starch content and velocity of water uptake and an inverse relationship between nitrogen content and imbibition rate was reported. The surface layers of the barley kernel are the principle barriers to water entry. The pericarp and testa are the major organs for regulating water entry. Variation of temperature during imbibition influences the rate of water uptake by the barley seed. All barleys, however, have a similar temperature coefficient of imbibition.

Briggs (1978) reported smaller kernels take up moisture faster and to a higher final level than larger kernels. Genotype differences for the water uptake were observed using Compana isotypes (Bruckner, 1981). Naked and waxy isotypes were found to take up water faster than normal isotypes in a 48 hour, 20 C, unaerated steep.

Barley Milling

The purpose of modern flour milling in wheat is to separate the bran and germ from the endosperm, and to reduce the size of the endosperm particles through a series of milling steps. Bran and germ are deleterious in baking performance and may be less digestible.

Flour extraction is defined as the proportion of flour by weight, obtained by milling a known quantity of wheat. It has been used as an index of the overall efficiency of a flour milling enterprise. Since the ash content at a given flour extraction varies within a narrow range, ash % has been used as an indicator of flour mill performance. As extraction increases, ash content of the flour increases. Percent ash in the flour reflects the efficiency of separation of bran from endosperm (Pomeranz, 1971).

Pomeranz et al. (1971) described a method for milling barley to increase the flour extraction up to 65 percent. The barley was tempered for 30 minutes with 0.5% water added before milling, red dog and shorts were reground on an alpine mill and sifted through a 100 xx sieve, and the throughs, called tailings flour, were mixed with patent flour.

McGuire (1979) developed the Buhler milling process for barley changing several steps of the wheat milling method to extract suitable flour from covered and naked barley. Barley was tempered to 13% moisture for 30 minutes before milling and the 145 mesh flour sieves were replaced with 88 mesh sieves. Shorts were returned to first break rolls

for a second passage through the mill. The feed rate for whole grain to first break was about 75 g/min.

Cheigh et al. (1975) tempered naked barley for 48 hours to a moisture level of 13.5%. An addition of 0.5% more water prior to flour milling increased the milling efficiency compared to 30 minutes tempering to a 14% moisture level.

Sorum (1977) reported that the dry milling of barley produced higher average flour extraction without increasing significantly the ash content in the flour over milling tempered barley. Because of the soft woolly nature of the barley endosperm, too long and high moisture tempering tend to produce crushed or flaked barley at the first break roll (McGuire, 1979).

Flour extraction of barley varied between 50 and 74% depending on the milling method applied and upon the samples used (Cheigh et al., 1975; Sorum, 1977). McGuire (1979) found an average extraction of 65% from Steptoe and a high extraction of 72.2% from Betzes.

Cheigh et al. (1975) indicated that use of hulless barley cultivars resulted in approximately 10% greater flour extraction than that from covered barley cultivars, without increasing ash content in the flour.

Ash content in the barley flour from milling experiments ranged from 1.23 to 1.98% on a dry basis. Positive relations between whole grain and flour protein ($r=0.763^{**}$), and whole grain and flour ash ($r=0.426^{**}$) indicated that the flour characteristics reflected the variability of the grain among cultivars (McGuire, 1979).