

## AN ABSTRACT OF THE THESIS OF

Chris Weight for the degree of Master of Science in Crop Science presented on December 12, 1996.

Title: Possible Associations Between Three Procedures To Measure Noodle Starch Quality in Winter Wheat (*Triticum aestivum* L.)

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Until recently, the viscoamylograph had been the primary method for measuring starch as it relates to noodle quality in wheat. This method requires a large sample of flour and is time consuming. With the introduction of the rapid visco-analyser (RVA) in 1986 and the more recent flour swelling volume procedure, both the time to determine starch quality and the sample size required have been reduced

Parents, reciprocal  $F_2$ , and backcross generations from a cross involving two white wheat lines were space-planted to study the association among the three procedures for measuring noodle starch quality. Grain protein and hardness and plant height also were measured. Differences among the generations were observed for the viscoamylograph, flour swelling volume, and plant height. Coefficients of variation were low for both flour swelling volume and plant height.

A significant positive association was found between the viscoamylograph and the RVA ( $r=0.61$ ). This indicates that for the populations evaluated in this study the RVA can be used in place of the viscoamylograph to measure starch quality. The RVA values, however, varied greatly between years suggesting a large environmental influence. There was also evidence for a genotype by environment interaction. No maternal effects were noted between reciprocal crosses.

Conflicting results were found for the association between the flour swelling volume procedure and the RVA and viscoamylograph. Further experiments are required to clarify the possible association between flour swelling volume and other starch quality tests. Such experiments may have to be delayed until the  $F_4$  or  $F_5$  generations when adequate amounts of flour can be obtained from more genetically uniform lines.

Possible Associations Between Three Procedures to Measure Noodle Starch  
Quality in Winter Wheat (Triticum aestivum L.)

by

Chris Weight

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# **POSSIBLE ASSOCIATIONS BETWEEN THREE PROCEDURES TO MEASURE NOODLE STARCH QUALITY IN WINTER WHEAT (Triticum aestivum L.)**

## **INTRODUCTION**

Wheat has been an important part of the Asian diet for over 1000 years. Noodles, which are primarily made from white wheat, have been one of the major end-products of wheat in Asian countries. Many different kinds of noodles are produced in Asia due to the unique preferences found in each country. This thesis will focus on Japanese and Oriental noodles.

In recent years Asian countries are primarily importing wheat for the production of noodles from Australia. This type of wheat, which is white wheat, also is produced in the Pacific Northwest. Historically, this area had a large percentage of the Asian noodle market. To be more competitive, wheat breeders in the Pacific Northwest would like to determine what makes the Australian wheat more desirable for noodle production. With this information, breeders can then focus on increasing the quality of wheat used for noodle production.

Starch, which makes up 70% of wheat flour, is the most important constituent of wheat for noodle production. Several different procedures are used to determine starch quality in this regard. These procedures are the viscoamylograph, rapid visco-analyser (RVA), and flour swelling volume. The viscoamylograph is the standard instrument for measuring starch quality.

This instrument is used by most Asian countries to determine flour quality. This procedure is relatively slow and requires a large sample of wheat flour (100g). The other procedures, the rapid visco-analyser and flour swelling volume, represent efforts to speed up starch quality measurements as well as reduce the sample size required.

All three procedures are based on the amount of water absorbed by starch granules during gelatinization. The viscoamylograph and RVA measure peak viscosity of the starch paste as the amount of resistance to stirring. Flour swelling volume is an actual measurement of how much the starch has swelled after gelatinization due to the uptake of water.

If these three procedures could be used interchangeably, flour swelling volume, which is the simplest and quickest for determining noodle starch quality, would be more useful to breeders. Determination of starch quality in  $F_2$  and  $F_3$  selections would be possible instead of waiting until later generations when larger seed samples are available. This would make wheat breeding for starch quality much more efficient and cost effective.

Therefore, the objectives of this study were to identify a reliable procedure for starch quality determination for use during the early generations of breeding and to determine the possible associations among three procedures which measure noodle starch quality.



## LITERATURE REVIEW

While rice has historically been the chief component of the Japanese diet, wheat also has played an important role in their diet in one form or another since the second century A.D. (Nagao, 1995). The current per capita consumption of wheat in Japan is 32 kg (Nagao, 1991 and Miskelly, 1996) with the total wheat consumption in Japan at six million tons annually. Of this amount, 85% is imported (Konik et al., 1990) with the United States being the largest supplier at 57% of total wheat imports. Canada, at 26%, and Australia, at 17%, are the only other significant exporters of wheat to Japan. Bread and confectionery products are the main end uses of wheat imported from the U.S. and Canada. Noodles are primarily made from Australian wheat (Nagao, 1995).

Noodles have been a part of the Japanese diet for over 1000 years. They were first introduced into Japan from China and have been modified several times to suit the preferences of the Japanese. There are many different types of noodles but all of them consist of at least three main ingredients. These ingredients include flour at 100 parts, salt at 2 to 3 parts, and water at 28 to 45 parts (Nagao, 1991, Nagao, 1995, Oh et al., 1985b, and Crosbie et al., 1990). White noodles limited to these ingredients are known as Japanese noodles and are not common outside of Japan (Miskelly and Moss, 1985). Nagao (1995) states that "Japanese noodles are creamy

white in appearance and soft in texture. They are classified by width into very thin (so-men), thin (hiya-mugi), standard (udon), and flat (hira-men)". These noodles also can come in many forms such as fresh (raw) noodles, boiled (wet) noodles, dry noodles, fried noodles, instant fried noodles, and instant dry noodles (Oh et al., 1983 and Hoseney, 1994). Kim (1996) notes that there are many different kinds of noodles in production throughout Asia. These include wheat (Japanese and Chinese-type) noodles, buckwheat noodles, starch noodles, and rice noodles. Due to the simple formula for noodles, flour is the key quality determinant (Nagao, 1991). Kim (1996) defines flour quality as "the ability of the flour to produce a uniformly good end-product under conditions agreed to by the supplier and the customer". Kim also notes that flour quality is relative in that expectations can vary widely from one country to another, and from one product to another.

The flour type usually used for Japanese noodles comes from soft-grained white wheat (Crosbie et al., 1990) imported from Australia. About 70% of the 900,000 to 1,000,000 tons of Australian wheat imported by Japan each year is used for the production of Japanese noodles. This segment of wheat is known as Australian Standard White (ASW) (Konik et al., 1990, Crosbie et al., 1990, and Nagao, 1989). Historically, ASW has been a combination of several white wheats. Recently the bulk of this class consists of two wheat cultivars, 'Gamenya' and 'Eradu'. These cultivars are regularly segregated from other cultivars so that they can be shipped to

Japan for the production of Japanese noodles. The flour produced from these cultivars was determined by the Japanese as being close to ideal for the production of Japanese noodles (Konik et al., 1990 and Nagao, 1996).

About 30% of the wheat imported from Australia is known as Australian Prime Hard (APH). This segment of the wheat crop is used for the production of yellow Chinese-type noodles as well as various types of breads (Crosbie et al., 1990). Roughly 16% of all flour consumed in Japan each year goes toward the production of Chinese noodles with an equal amount being used for Japanese noodles (Crosbie, 1991). Chinese-type noodles have been modified to meet the tastes of the Japanese and have been popular in Japan for about 70 years (Nagao, 1991). In Japan, the Chinese-type noodles are called ra-men. These noodles, unlike Japanese noodles, can be found throughout East Asia with the quality varying from country to country. A typical formula for the production of Chinese-type noodles includes hard wheat flour at 100 parts, water at 32 to 35 parts, salt at 0 to 2 parts, and kansui at about 1 part (Nagao, 1995).

Kansui is a mixture of sodium phosphate, potassium carbonate, and sodium carbonate (Nagao, 1991). When kansui is added to the dough, a "yellow colour develops by the action of the alkaline salts on the natural flavonoid compounds in the flour" according to Crosbie et al. (1990). This yellow color needs to be light and uniform throughout the noodles in order to be accepted by the Japanese consumers (Nagao, 1995). Shelke et al.

(1990) notes that as the amount of sodium carbonate is increased, the yellow color of the noodles gets deeper. These salts affect pasting properties such as increasing the starch gelatinization temperatures (Huang and Morrison, 1988). Also, the addition of the alkaline salts usually toughens the dough and inhibits enzyme activity. This inhibitory action suppresses enzymatic darkening which, if left unchecked, could lead to undesirable colors in the noodles (Miskelly and Moss, 1985 and Moss et al., 1986).

The key ingredient in both types of noodles is flour. The quality of the noodle depends strongly upon the properties of the flour being used (Oh et al., 1985a). There are many factors to consider when measuring the quality of flour. This is apparent in the following statement made by Baik et al. (1994) in which he comments that the properties in flour related to oriental noodle production include: "1) wheat hardness and milling properties, such as particle size and flour yield; 2) starch characteristics, including amylose-amylopectin ratio, starch pasting properties, and swelling power; 3) protein content and protein quality parameters, such as sodium dodecyl sulfate sedimentation, mixograph, and alveograph values; and 4) color characteristics" such as discoloration and pigmentation. This statement makes it clear that the starch, which makes up at least 70% of the flour, must have certain characteristics to enable the production of quality noodles (Kruger, 1996).

## Starch

Starch, a carbohydrate that serves mainly to store sugars in plants (Mathews and vanHolde, 1990), is stored in the endosperm of wheat (Miura and Tanii, 1994). Starch is made up of granules which come in two sizes known as A and B granules (Morrison, 1989, Eliasson, 1989, and Shi and Seib, 1989). The A granules are lenticular in shape and vary in size from 20 to 35  $\mu\text{m}$ . The B granules are spherical in shape and vary in size from 2 to 10  $\mu\text{m}$ . Shi and Seib (1989) note that the B granules account for about 30%, by weight of starch.

The chemical composition and properties of these two types of granules are essentially the same (Hoseney, 1994) with only slight variations in the amount of amylose, amylopectin, lipid, and protein contents occurring between the granules (Shi and Seib, 1989). The amounts of proteins and lipids occur at such low levels in the granules, 1% or less (Galliard et al., 1989), that they are thought to simply be contaminants in the starch (Hoseney, 1994 and Shi and Seib, 1989). The two types of starch granules are made up of a six-carbon carbohydrate known as glucose. Glucose comes in two forms depending upon how the glucose units are chemically linked to each other. If the glucose units are linked together by alpha-1,4 bonds the polymer is known as amylose. If the glucose units are linked together by alpha-1,4 bonds and alpha-1,6 bonds the resulting branched polymer is known as amylopectin.

In addition to structure, the purpose of these two different polymers is different. Amylose is formed by the plant for long-term storage of glucose. Amylopectin is formed so that glucose can be mobilized rapidly when necessary (Mathews and vanHolde 1990). Shi and Seib (1989) noted that the A granules are the first to develop in the endosperm followed later by the B granules. Moss and Miskelly (1984) add that as a growing season progresses, the amylose component of starch increases more than the amylopectin component (Moss and Miskelly, 1984). According to Hosene (1994) "The ratio of amylose to amylopectin is relatively constant, at about  $23 \pm 3\%$  amylose" in mature seeds.

The ratio of amylose to amylopectin has been shown in several studies to affect the overall noodle quality; e.g. as the percent amylose content decreases, the quality of the noodles increases (Moss, 1980, Oda et al., 1980, Moss and Miskelly, 1984, Toyokawa et al., 1989b, Crosbie, 1991, and Miura and Tanii, 1994). Cultivars from Australia which make up ASW have very favorable amylose to amylopectin ratios when compared to cultivars from other countries (Moss, 1980). This might lead a scientist to develop the simple strategy of measuring the percent amylose content of wheat cultivars to help determine which cultivars should be used for the production of noodles; unfortunately, problems exist with this approach. The percent amylose content varies very little among the majority of wheat cultivars and percent amylose content is relatively constant over a wide

range of known starch qualities in different wheat cultivars (Crosbie, 1991 and Miura and Tanii, 1994). This meant that simply measuring the percent amylose content could not give a consistently clear indication of differences in starch quality. This has forced scientists to look for other ways of measuring starch quality.

### **Gelatinization**

Once starch is placed in water, the granules can be freely penetrated by water. This causes the granules to swell and their volume to increase. These changes are reversible unless the starch and water is heated to the point of gelatinization (50 to 57°C). Once the starch is past this point, all changes to the starch granules are irreversible.

Gelatinization of starch is defined as the loss of birefringence, a trait starch granules have due to their high degree of molecular order. Birefringence shows up when the starch granules are viewed in polarized light as a "maltese cross". Morrison (1989) notes that the loss of birefringence is most notable with the disordering of amylopectin crystallites.

As the solution of starch and water is heated past the point where gelatinization begins, the starch granules swell to the point of distortion. Soluble starch is then released into the solution and the granules continue to take up more water. These events lead to an increase in the viscosity of the

solution. This thickening property is used in the manufacture of many foods such as gravies and puddings (Hoseney, 1994).

The concept of starch gelatinization also is used in several tests as a way of determining the level of starch quality in flour. One instrument which can be used for this test is the Viscoamylograph. The Brabender Viscoamylograph is the oldest and most widely used instrument for testing starch quality (Ross et al., 1987 and Walker et al., 1988). In this test, flour and amylograph buffer are heated from 30°C to 95°C at 1.5°C per minute. This solution is stirred the entire time and the viscosity of the solution increases as the starch is gelatinized (Deffenbaugh and Walker, 1989). The viscosity of the solution will reach a maximum near 95°C and the measurement of viscosity at this point is known as the peak viscosity (Moss and Miskelly, 1984). This measurement is the most relied upon number for estimation of overall starch quality of flour.

Several studies (Kim and Seib, 1993, Nagao et al., 1977, and Nemeth et al., 1994) have shown that the samples with the highest noodle-making quality were those that reached peak viscosity quickest. This means that these samples were reaching their peak viscosity at lower temperatures than poorer samples. Kim (1996) noted that the amylograph peak viscosity could be altered by adding salt and/or alkali to the flour sample. The peak viscosity was increased in an additive manner when either or both of these ingredients were added. It is important to notice that both of these ingredients are used



in the production of Chinese noodles and at least one ingredient, salt, is used in the production of Japanese noodles. This allows for increased flexibility in choosing a source of flour for noodle production.

After the solution is heated to 95°C, this temperature is maintained for a period of time (from 10 to 60 minutes depending upon the protocol being used). The solution is then cooled at a rate of 1.5°C per minute back to 50°C (Deffenbaugh and Walker, 1989). This further testing yields additional data such as holding strength, breakdown, and final viscosity. Studies have shown that these measurements, which reveal starch pasting characteristics of the flour, also can be useful for determining noodle quality (Konik et al., 1992).

It can take over 2 hours to complete all of this testing for a single flour sample (Walker et al., 1988). The test also takes up to 100g of flour per test. These two facts have been major drawbacks for scientists who want to perform this test. Flour in the amount of 100g is not usually available in a breeding program until later stages of breeding after a lot of time and money has been spent getting selections to this point (Panozzo and McCormick, 1993). The two drawbacks made it clear that scientists needed to develop another method for determining starch quality. The Australians were the first to develop a new method which dealt with the drawbacks of the viscoamylograph.

In the 1983-84 Australian wheat harvest, more than 22% of the harvest was downgraded due to weather damage. This resulted in a loss of value between 23 and 32% (Ross et al., 1987). This weather damage was in the form of late rain near harvest which caused levels of alpha-amylase to increase in the wheat seed. This damaged wheat is called sprouted wheat and it possesses very poor starch quality resulting in inferior noodles and other products (Deffenbaugh and Walker, 1989, Nemeth et al., 1994, and Hosney, 1994). Kim (1996) adds that the use of sprouted wheat for the production of noodles can result in noodles which would be unmarketable. Zawistowska (1989) comments that "Degradation of carbohydrate storage reserves by alpha-amylase results in deterioration of flour quality for production of noodles." Crosbie and Lambe (1993) and Galliard et al. (1989) also noted that increased levels of alpha-amylase resulted in a lower viscosity for the gelatinized flour, thus reducing the quality of the flour.

After the huge losses of the 1983-84 season, the Australians set out to develop a test to check for sprout damage in wheat which was quick and easy, requiring minimal operator training. These requirements for the test were necessary due to the grain receiving procedures in Australia (Ross et al., 1987 and Batey et al., 1989).

Tests, such as the Hagberg Falling Number method and the Amylograph test were already available. These tests could indirectly determine the amount of alpha-amylase present in the seed (de Francisco et

al., 1989). The problem was that these tests were either too time-consuming, delicate, or complex for practical use during the grain receiving period (Ross et al., 1987).

An instrument called the Rapid Visco-Analyzer (RVA) was developed jointly by the Australian CSIRO Wheat Research Unit and Bread Research Institute for use during the 1986-87 wheat harvest. Batey et al. (1989) noted that the RVA was designed to measure the amount of resistance to stirring a gelatinized mixture of water and wholemeal. Ross et al. (1987) found a 95% correlation between the results of the RVA and the Falling Number method which suggested that the RVA was a reliable test for sprout damage.

Subsequent modifications to the RVA proved that it was useful not only for sprouting damage tests, but also for revealing the starch pasting characteristics of flour. The RVA provided results, including the peak viscosity, using a much smaller sample size (only 4 grams of wholemeal) and in much less time (only 15 to 20 minutes depending upon the protocol being used) (Deffenbaugh and Walker, 1989, Batey et al., 1989, and Walker et al., 1988). It is also important to note that wholemeal is used with the RVA rather than flour which is required for the viscoamylograph test. This further magnified the convenience of the RVA and its' suitability as a replacement for the viscoamylograph.

Testing of the two instruments revealed that the starch quality results given by the two instruments were highly correlated ( $p < .01$ ) (Panozzo and McCormick, 1993). Testing also showed that wholemeal could be reliably used with the RVA to give consistent results (Konik et al., 1994). This proved that the RVA parameters, such as peak viscosity, could successfully be used to predict noodle quality (Panozzo and McCormick, 1993 and Konik et al., 1990).

With the invention of the RVA, scientists could now test 25 to 30 samples a day, up from just a few samples a day using the viscoamylograph. This was a great improvement, but room for improvement still existed. Crosbie et al. (1992) noted that "the measurement of the extent of swelling of starch or flour during gelatinization may be the basis for a simple test that would discriminate among early generation lines on their potential suitability for making Japanese noodles." Konik et al. (1993) and McCormick et al. (1991) also noted that simply measuring the swelling of the starch granules caused by the uptake of water during heating could possibly be used to predict starch quality.

With this research, a new test called the starch swelling power test was developed. This test had the main advantages of requiring less than 1 gram of wholemeal and being fast. As many as 100 samples a day could be run by one person using this method (Crosbie et al., 1991). These advantages made it much more practical to test early generation lines where

only small samples are available (Panozzo and McCormick, 1993 and Crosbie et al., 1992).

The swelling power test has been shown to be highly correlated ( $p < 0.001$ ) with the amylograph peak viscosity (Baik et al., 1994 and Crosbie et al., 1991). It also has been shown to be highly correlated ( $p < 0.01$ ) with the RVA peak viscosity which indicated that the starch swelling power test could be used to predict the potential noodle quality of samples during early generations of a wheat breeding program (McCormick et al., 1991).

### **Color**

A very important factor for determining noodle quality is color (Morris, 1994, Lee et al., 1987, and Miskelly and Moss, 1985). Konik et al. (1994) notes that "noodle colour and brightness were found to be adversely affected by increased protein content, brown and yellow pigments, presence of any non-endosperm material, and loss of reflecting power by damaged starch granules." Miskelly (1984) adds that wheat cultivars and milling extraction rates could add to differences in the appearance of noodles. Lee et al. (1987) and Oh et al. (1985c) also showed that the extraction rate used during the milling process affects the flour color. As the flour extraction rates were increased (above 60%), the color of the noodles made from this

flour got darker which decreased overall noodle quality. If the extraction rate is too high, the noodles become unacceptable to Asian consumers.

Another result of increasing extraction rates is increasing levels of enzymatic browning in noodles. Oh et al. (1985c) explains that the amount of bran particles increases with increasing extraction levels. These bran particles contain high concentrations of pigments and oxidative enzymes. One of these enzymes, polyphenol oxidase, is responsible for the darkening of noodles during storage (Kruger, 1996 and Hoseney, 1994). This darkening can be controlled by boiling the noodles at the time of production which denatures the enzyme (Hoseney, 1994 and Miskelly, 1984).

Pigments contained in the bran particles can also lead to undesirable colors if they are not controlled. Flavonoid pigments found in the bran particles can cause yellowing of the noodles. This is taken advantage of with the use of alkali for the production of Chinese noodles, but this color is undesirable for Japanese noodles (Hoseney, 1994).

Starch damage, which takes place during the milling process, has been shown by Konik et al. (1994), Miskelly (1984), and Oh et al. (1985c) to produce a dark color in noodles. They found a significant negative correlation between starch damage and flour brightness. Miskelly (1984) attributed this to the fact that whole starch granules reflect more light than damaged starch granules. As the amount of starch damage goes up, the amount of light reflected goes down leading to the production of duller flour

color. Panozzo and McCormick (1993) and Craig (1989) commented that softer wheats usually suffer lower levels of starch damage than harder wheats. This is one reason why soft wheats are preferred for Japanese noodles instead of harder wheats (Konik et al., 1992 and Nagao, 1996).

The color of noodles is also closely linked with protein contained in the flour. Several authors (Lee et al., 1987, Rho et al., 1989, Shelke et al., 1990, Konik et al., 1994, Miskelly, 1984, and Miskelly and Moss, 1985) have reported that as protein increased, the flour brightness decreased. Oh et al. (1985c) explains that "Flour protein may produce a tight noodle structure resulting from a strong adherence between starch and protein. Such a tight structure would cause uncooked noodles to appear translucent, resulting in less reflected light in high-protein noodles."

Even with this knowledge, scientists do not breed exclusively for low protein wheat cultivars in order to maximize flour brightness. This is due to the positive correlation existing between protein content and firmness of the noodle (Konik et al., 1994) which is one of several factors included in the category of noodle texture. Shelke et al. (1990) found this correlation to be highly significant ( $p < 0.001$ ). Hoseney (1994) notes that noodles made from flour with a low protein content have a poor cooking tolerance. These noodles tend to become mushy when overcooked making the noodles unacceptable.

This positive correlation between protein content and firmness, along with the negative correlation between protein content and flour brightness caused noodle manufacturers to specify protein content ranges for the wheat they import. An optimum range has been established which maximizes brightness and textural qualities (Miskelly and Moss, 1985 and Konik et al., 1993). Australian Standard White wheat at protein levels between 10.5% and 10.7% and APH wheat above 13.0% protein content have proven to be acceptable levels for producing bright noodles with good texture (Crosbie et al., 1990).

### **Taste and Texture**

Taste and texture are the last major considerations for determining overall noodle quality. The taste of noodles is subjective with any sour, strange, or bitter tasting noodles rejected (Toyokawa et al., 1989a).

Texture consists of many distinct factors which, when combined, give an overall measure of the textural quality of the noodle. The major considerations of texture are 1) the balance between softness and hardness 2) smoothness and 3) viscoelasticity (Nagao, 1996, Oh et al., 1985b, Crosbie et al., 1990, and Toyokawa et al., 1989a).

The balance between softness and hardness gives a measure of how firm a noodle is. Crosbie et al. (1992) notes that the noodles need to be soft, but not mushy. They also need to possess a slight amount of surface



firmness. Konik et al. (1994) and Oh et al. (1985c) reported that starch damage decreases the firmness of noodles. Oh et al. (1985b) showed that the firmness of noodles also decreases when the noodles are cooked in alkaline water.

Smoothness, which is the second consideration of texture, requires a small degree of gluten development for optimum quality (Nemeth et al., 1994 and Rho et al., 1988). If gluten development, which is achieved during the sheeting steps of noodle manufacture, is too high (Oh et al., 1985a) or too low (Oh et al., 1985b), the smoothness of the noodle goes down reducing the overall quality of the noodle.

The last component of texture, viscoelasticity, is considered the most important part of texture (Nagao, 1996, Crosbie et al., 1992, and Toyokawa et al., 1989a). These authors note that consumers want a high degree of elasticity or springy resistance but not too much resistance. Baik et al. (1994), Miura and Tanii (1994), Hoseney (1994), and Panozzo and McCormick (1993) found that as the amount of protein in the flour increased, the chewiness (or resistance) of the noodle increased. Toyokawa et al. (1989a) went on to say that the quality of the protein, not just the protein content, influenced the viscoelasticity of the noodle. Konik et al. (1994) adds that as the level of starch in the flour goes up, the elasticity of the noodle goes down.

In the past, scientists have employed the use of trained panels for sensory evaluation of noodles. Oda et al. (1980) points out that this method of evaluation is unreliable due to the subjective nature of this form of testing. An objective, reproducible test is required for reliable measurement of the textural properties of noodles.

Baik et al. (1994) and Oh et al. (1983) have shown that a texture profile analysis can be obtained using the Instron Universal Testing Machine. This machine, with the help of other tests which determine starch and noodle quality, will hopefully aid scientists in their efforts towards breeding high quality wheat for the production of noodles.

## MATERIALS AND METHODS

Two selections from the 1993 winter wheat quality crossing block established at the Hyslop Crop Science Field Laboratory near Corvallis, OR, were selected as the parents. OR #890214 (Agric/BJYS//VEES), a semi-dwarf hard white winter wheat, and OR #880485 (OR 7946/HILL//HILL), a semi-dwarf soft white winter wheat were the parents. Selection of the experimental material was based upon the starch quality as measured using an RVA. In 1993, OR #880485 (parent 1) had a medium RVA viscosity of 163 Stirring Number Units (SNU) and OR #890214 (parent 2) had an RVA viscosity value of 283.

### Study I 1994

The parents and their reciprocal crosses were planted at Hyslop on October 28, 1993. The soil type at this site is a Woodburn silt loam. Prior to planting, 45 kg/ha of urea (46-0-0) were applied. A randomized block design with four replications was employed. 'Rohde', a club wheat, was used as a border around this experiment to avoid any border effect. Within each replication two blocks were established with parents and one reciprocal  $F_1$  in block one and the parents and the second reciprocal  $F_1$  in block two. Each block contained four space-planted rows 30cm apart with 1 row for each parent and 2 rows for the reciprocal  $F_1$  cross. Each row consisted of

10 plants spaced 30cm apart. Weeds were controlled with a fall application of Diuron (dichlorophenyl) at a rate of 1.1 kg AI/ha and by hoeing. A total of 28 kg/ha of nitrogen and 5.6 kg/ha of sulfur were applied at tillering (Feekes stage 4) in the form of 30-0-0-6 fertilizer (ammonium nitrate-sulfate).

Reciprocal backcrosses were made in the spring of 1994 and the experiment was harvested on July 25, 1994.

### **Study II 1995**

The material from 1994 was planted at the Crop Science Field Laboratory on November 2, 1994 in a randomized block design with four replications. Prior to planting, 45 kg/ha of urea (46-0-0) was applied. The cultivar 'Gene' was used as a border around this experiment. Within each replication there were two blocks. Block one contained the parents,  $F_2$ 's, and four backcrosses from one reciprocal  $F_1$  while block two contained the parents,  $F_2$ 's, and four backcrosses from the other reciprocal  $F_1$ . Each block contained 16 space-planted rows 30cm apart with 2 rows for each parent, 4 rows for the  $F_2$ 's, and 2 rows for each of the four reciprocal backcrosses. Each row consisted of 25 plants spaced 18cm apart. Reciprocal backcrosses were made to see if maternal effects existed which could influence the procedures and traits under investigation. A flow diagram explaining how the generations were developed is given in Appendix Table 1.

Weeds were controlled by hoeing. A total of 28 kg/ha of nitrogen and 5.6 kg/ha of sulfur were applied at tillering (Feekes stage 4) in the form of 30-0-0-6 fertilizer (ammonium nitrate-sulfate).

Plant height (measured from the soil surface to the tip of the spikes, excluding the awns) was measured for each row and on individual F<sub>2</sub> plants.

After harvesting, a sample of grain was ground using an udy cyclone mill with a 0.5mm mesh screen to obtain wholemeal. Approximately 71.5g of grain from each sample was also sent to Pullman, WA. and ground in a modified Quadramat Senior mill to obtain flour. Before milling, these samples were tempered to a 13.7% moisture content.

The wholemeal was used in determining grain protein content and grain hardness using near-infrared reflectance spectroscopy with a Technicon Infralyser 400. Part of this wholemeal was also used to perform starch quality tests using a Rapid Visco-Analyser (RVA). The RVA procedure was similar to the method used by McCormick et al. (1991). Twenty-five milliliters of distilled water were added to an RVA canister followed by 4 grams of wholemeal. The contents of the canister were gently mixed with an RVA paddle by moving the paddle up and down in the canister 10 times to break up clumps of wholemeal. The canister and paddle were then placed in the RVA. The suspension was first heated from 50°C to 60°C in 2 minutes and then heated up to 95°C during an additional 2 minutes and this temperature was maintained for 4 minutes. The suspension was then cooled

from 95°C to 50°C in 4 minutes. Peak viscosity was recorded from the digital display for each sample.

The wholemeal samples were also used to perform a flour swelling volume test. The flour swelling volume test is a modification of the starch swelling power test. The modifications include using wholemeal (0.5g) instead of isolating starch and calculating the swelling volume from the height of the gel in the tube instead of removing the gel from the tube and weighing it. These modifications have made the flour swelling volume test a much simpler, quicker test relative to the starch swelling power test for determining the suitability of flour for the production of noodles.

The flour swelling volume method was carried out according to the method used by Crosbie and Lambe (1993) with the following exceptions. Only 10ml of distilled water (5ml per step) was used in each tube instead of 12.5ml of water (6.25 ml per step) due to the smaller (100mm x 16mm instead of 125mm x 16mm) tube size. The 25°C water bath was eliminated and the addition of the  $I_2/KI$  solution to each sample was found to be unnecessary when proper back-lighting was used for each sample during the final measurement.

The amylograph procedure was carried out for the flour samples (using pin sensors) using a Brabender Viscoamylograph. The amylograph results were based on individual plant samples. This limited the amount of flour used in this procedure to 45g per sample rather than 60g of flour which is

normally required by most laboratories for determining starch quality with an amylograph. Forty-five grams of flour and 212ml of distilled water were combined in a 1-L Erlenmeyer flask. The flask was gently inverted 50 times and the flour slurry was poured into the amylograph bowl. The flask was then rinsed with an additional 100ml of distilled water and this was added to the amylograph bowl. The heating cycle was similar to the method used by McCormick et al. (1991) in which the amylograph was set to 30°C and increased to 95°C at a rate of 1.5°C per minute. After the heating period, the temperature of the suspension was maintained at 95°C for an additional 10 minutes and the test was ended at this point. Peak viscosity was recorded for each sample.

### **Analytical Procedures**

1. An analysis of variance was calculated for each procedure and trait to determine if differences existed between the generations.
2. Least square means were calculated for each trait. Fisher's Protected Least Significant Difference (FPLSD) was used to identify any significant differences between the least square means for each trait.
3. Phenotypic correlation coefficients were calculated between each trait.

## **RESULTS**

The results will focus on the following procedures and traits: 1)Rapid visco-analyser (RVA), 2)Viscoamylograph, 3)Flour swelling volume, 4)Grain protein, 5)Grain hardness, and 6)Plant height. Traits were measured for the parents,  $F_2$ , and backcross generations derived from a reciprocal cross on an individual plant basis.

### **Analysis of Variance**

Significant differences were observed among the generations for the viscoamylograph, flour swelling volume, and plant height (Table 1). The coefficient of variation was high for the viscoamylograph (29.42%) and low for the flour swelling volume test (5.39%) and plant height (2.93%). Coefficients of variation ranged from intermediate for grain protein (8.53%) and the RVA (12.88%) to very high for grain hardness (41.20%).

### **Separation of Means**

As the blocks in this analysis were unbalanced least square mean values were used to ensure that each block was weighted equally. The means were analyzed using Fishers' Protected LSD (FPLSD) to determine if significant differences existed for the measurements among the generations.



Table 1. Observed mean squares for six measurements involving parents, reciprocal F<sub>2</sub>, and reciprocal backcross generations obtained in 1995.

Source of variation	df	RVA (SNU) <sup>1</sup>	Visco-amylograph (BU) <sup>2</sup>	Flour swelling volume (ml)	Grain protein (%)	Grain hardness (NIR Score) <sup>3</sup>	Plant height (cm)
Replications	3	619.65	44029.88	0.93	0.35	3943.77	5.69
Generations	5	449.53	46596.31 *	1.21 *	1.44	956.81	98.27 **
Error	36	412.17	13101.80	0.35	0.90	437.70	9.49
Total	44						
C.V.		12.88	29.42	5.39	8.53	41.20	2.93

\*, \*\* Significant at the 0.05 and 0.01 probability levels, respectively.

<sup>1</sup> Stirring number units

<sup>2</sup> Brabender units

<sup>3</sup> Near-infrared reflectance score

Generation means of the procedures and traits are shown in Table 2.

The FPLSD test was not carried out for the RVA, grain protein, or grain hardness as the mean values were not significantly different.

Parent 1 had a much higher viscoamylograph mean relative to parent 2 in 1995. This relationship was opposite to what was expected based upon previous knowledge of the starch quality of these two parents. In 1993 and 1994 parent 2 had a higher level of starch quality than parent 1. Both of the  $F_2$  population means were lower than parent 2 in 1995. Backcrosses to either parent resulted in viscoamylograph means similar to parent 2.

Parent 1 also had a higher flour swelling volume mean than parent 2. Both of the  $F_2$  population means were between the values of parent 1 and parent 2. Backcrosses to either parent also resulted in flour swelling volume means between the parental values.

Parent 1 and 2 had similar plant height mean values. Both  $F_2$  population means were much higher than either parent. Backcrosses to either parent also resulted in plant height means higher than either parent but lower than either of the  $F_2$  population mean values.

Maternal effects were not observed in the mean values as no reciprocal differences existed. Therefore, the populations in this study were bulked to compare possible associations existing between the procedures and traits using phenotypic correlations.

Mean values and standard deviations of each trait for the generations are given in appendix table 2.

Table 2. Comparison of least square means performance for the parents, reciprocal  $F_2$ , and reciprocal backcross generations for the Rapid Visco-analyser, Viscoamylograph, Flour swelling volume, Grain protein, Grain hardness, and Plant height values obtained in 1995 using Fisher's protected LSD (FPLSD).

	RVA	Visco-amylograph	Flour swelling volume	Grain protein	Grain hardness	Plant height
Generation	(SNU)	(BU)	(ml)	(%)	(NIR Score)	(cm)
OR#880485	167.17 <sup>1</sup>	546.50a <sup>2</sup>	11.91d	10.37	32.82	97.66k
OR#890214	150.42	380.73abc	9.94f	12.52	70.87	98.98jk
$F_{2A}$ <sup>3</sup>	161.88	277.26c	11.13de	11.33	37.99	107.71gh
$F_{2B}$ <sup>4</sup>	140.94	266.89c	10.71ef	10.71	66.27	110.52g
BC/OR#880485	161.38	399.02b	11.26de	11.02	41.03	105.10hi
BC/OR#890214	164.47	377.15bc	11.09e	11.21	49.89	103.78ij

<sup>1</sup> FPLSD was not conducted, as the means were not statistically different

<sup>2</sup> Generation means which have common letters are not significantly different

<sup>3</sup>  $F_2$  populations originating from the cross OR#880485/OR#890214

<sup>4</sup>  $F_2$  populations originating from the cross OR#890214/OR#880485

### **Phenotypic Correlations**

Phenotypic correlations between all six procedures and traits measured are given in Table 3. The RVA and viscoamylograph tests showed a positive correlation. A negative correlation was observed between the flour swelling volume and both grain protein and grain hardness. Correlation coefficients between other measured variables were not significant.

Figure 1 illustrates the relationship between the RVA and the viscoamylograph. This graph shows a highly significant ( $p < 0.01$ ) positive association between these two procedures. The association between flour swelling volume and grain protein is shown in Figure 2. A highly significant negative correlation can be seen from this graph. The relationship between grain hardness and flour swelling volume (Figure 3) was also negative ( $p < 0.01$ ).

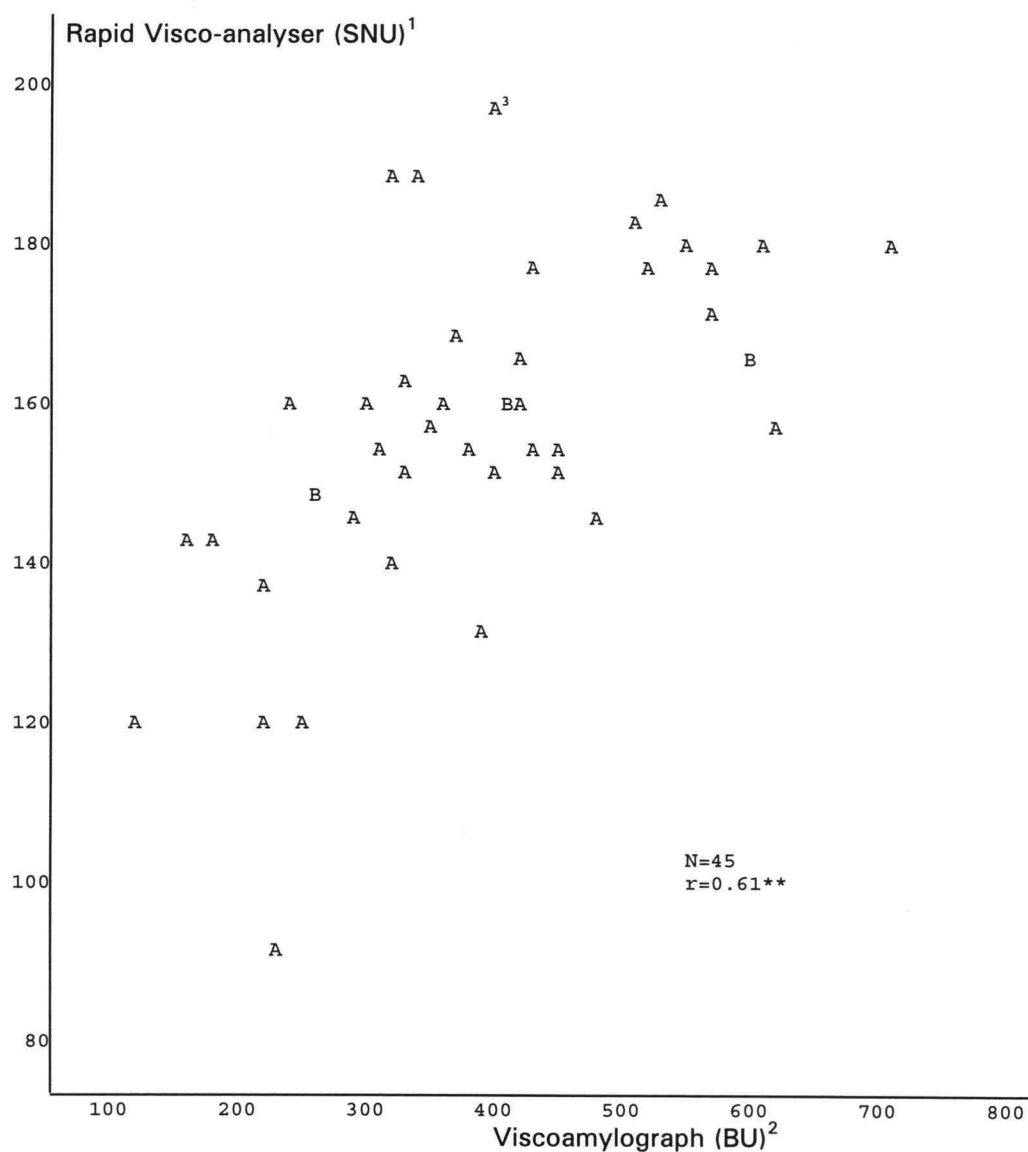
### **Frequency Distributions**

Figure 4 is a histogram of the frequency distributions of RVA values of the reciprocal backcross populations. The RVA peak viscosities are skewed towards the higher values with individuals being observed both above the high parent (parent 1) and below the low parent (parent 2). A histogram of the frequency distributions of viscoamylograph values of the reciprocal backcross populations are shown in Figure 5. The most notable feature of

Table 3. Phenotypic correlation coefficients between six selected procedures and traits measured on the parents, reciprocal F<sub>2</sub>, and reciprocal backcross generations obtained in 1995.

Correlation Coefficients						
Variable 1	RVA	Visco-amylograph	Flour swelling volume	Grain protein	Grain hardness	Plant height
Variable 2	(SNU)	(BU)	(ml)	(%)	(NIR Score)	(cm)
RVA	1.00	0.61 **	0.22	-0.06	-0.22	-0.16
Viscoamylograph		1.00	0.17	0.09	-0.04	-0.23
Flour swelling volume			1.00	-0.53 **	-0.74 **	-0.14
Grain protein				1.00	0.20	0.16
Grain hardness					1.00	0.04
Plant height						1.00

\*\* Significant at the 0.01 probability level (n = 45)



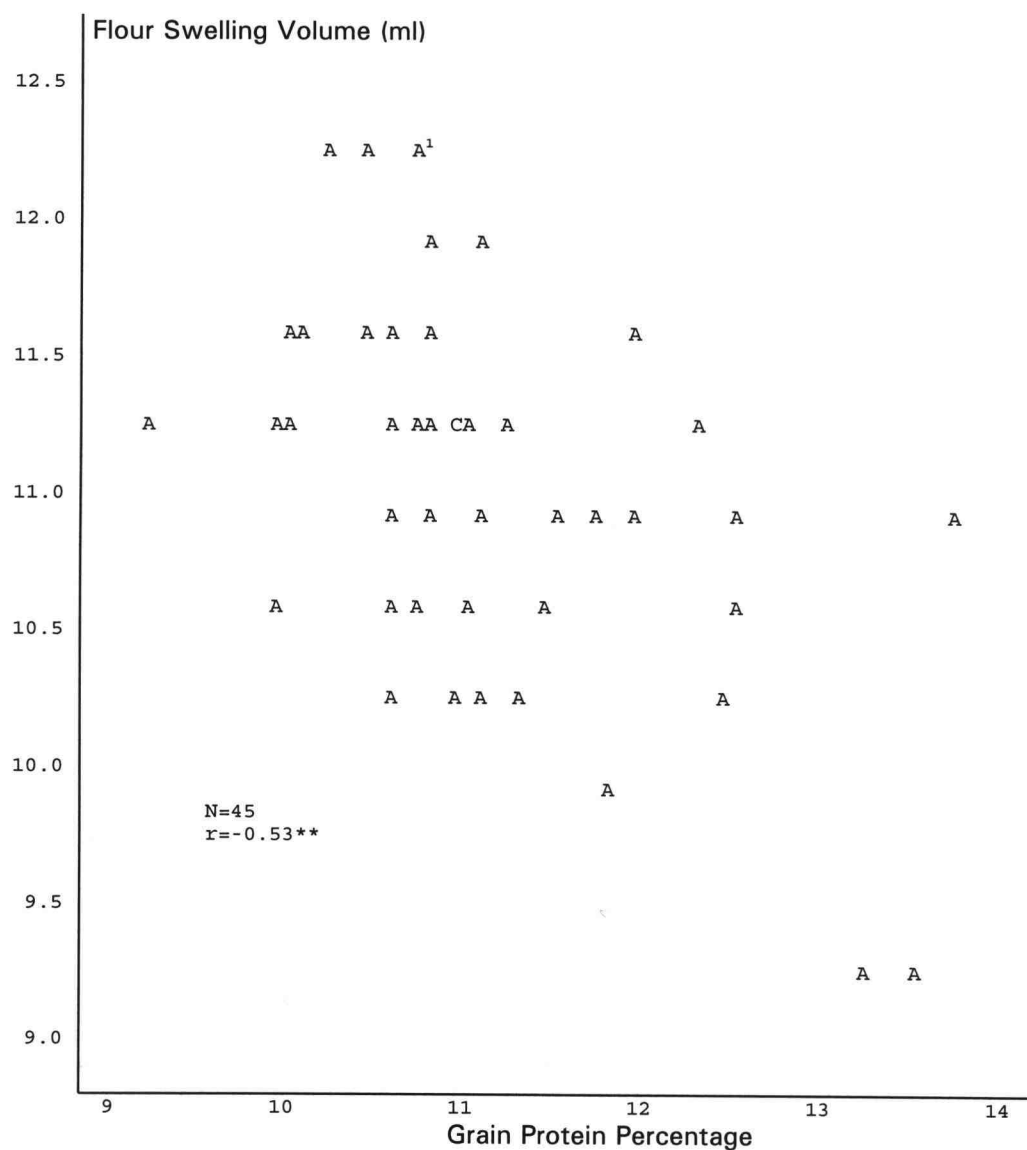
\*\* significant at the 0.01 probability level

<sup>1</sup> Stirring number units

<sup>2</sup> Brabender units

<sup>3</sup> A = 1 plant and B = 2 plants with the same values

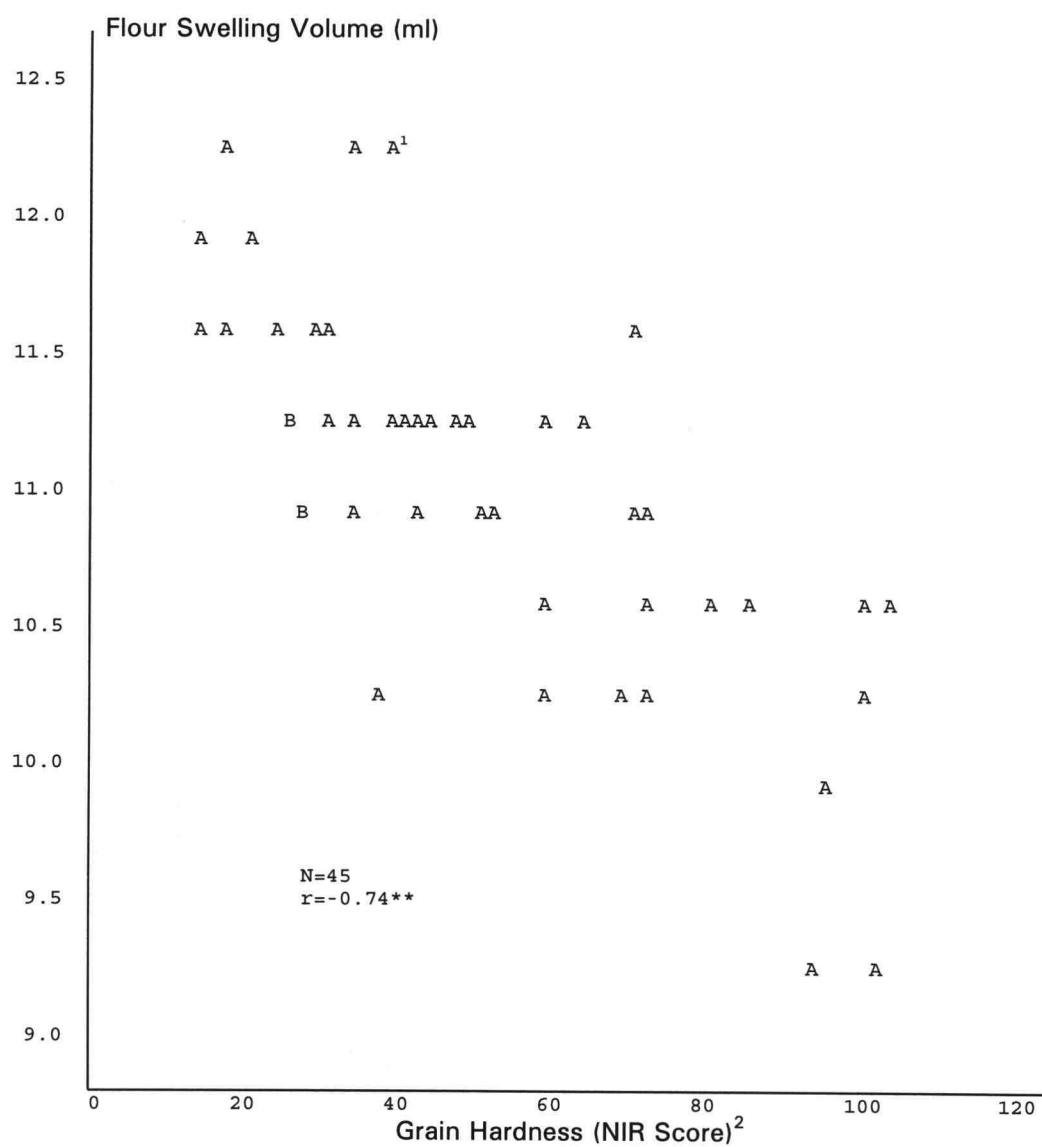
Figure 1. Relationship between the Viscoamylograph and the Rapid Visco-analyser.



\*\* significant at the 0.01 probability level

<sup>1</sup> A = 1, B = 2, and C = 3 plants with the same values

Figure 2. Relationship between flour swelling volume and grain protein.



\*\* significant at the 0.01 probability level

<sup>1</sup> A = 1 plant and B = 2 plants with the same values

<sup>2</sup> Near-infrared reflectance score

Figure 3. Relationship between flour swelling volume and grain hardness.



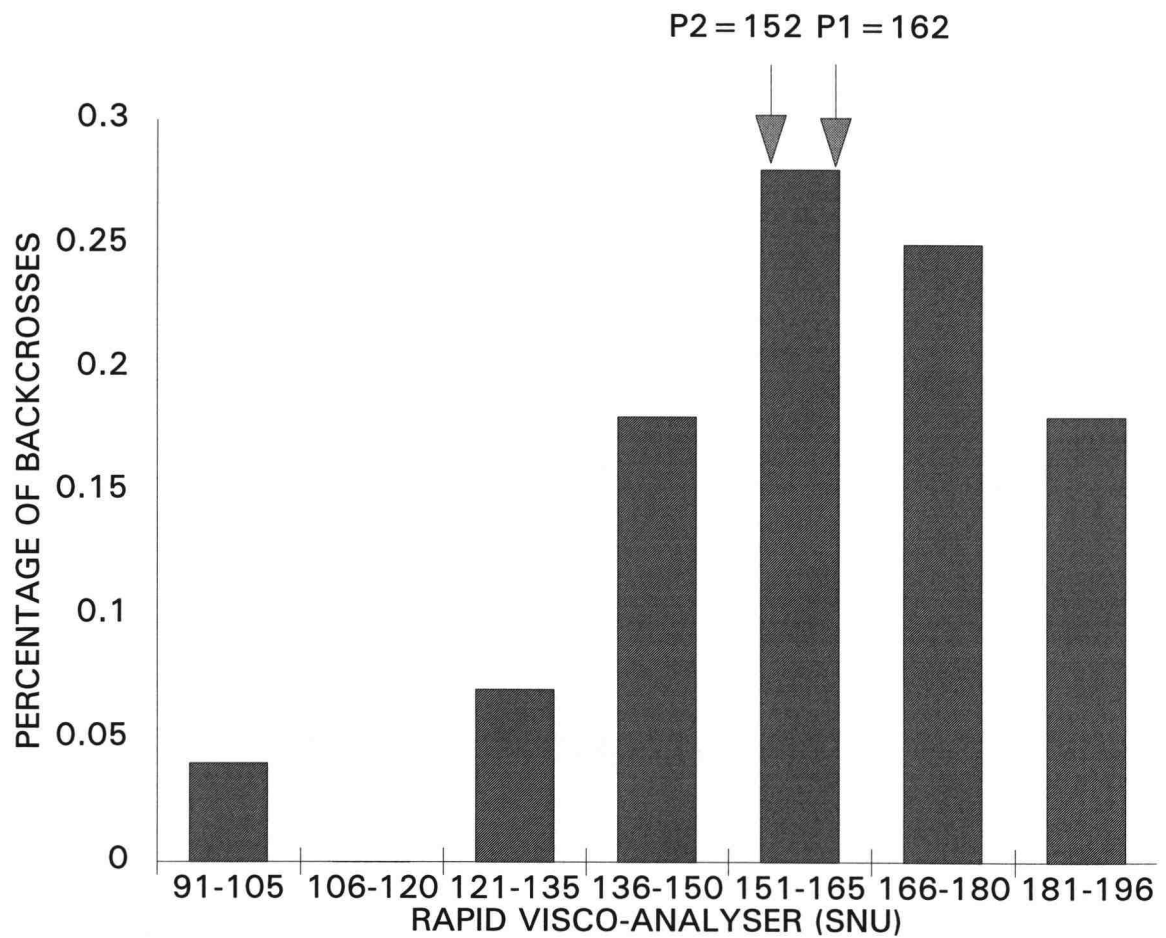


Figure 4. Frequency distributions of rapid visco-analyser values measure on reciprocal backcross populations ( $n=28$ ) and mean values OR#880485 (P1) and OR#890214 (P2) obtained in 1995.

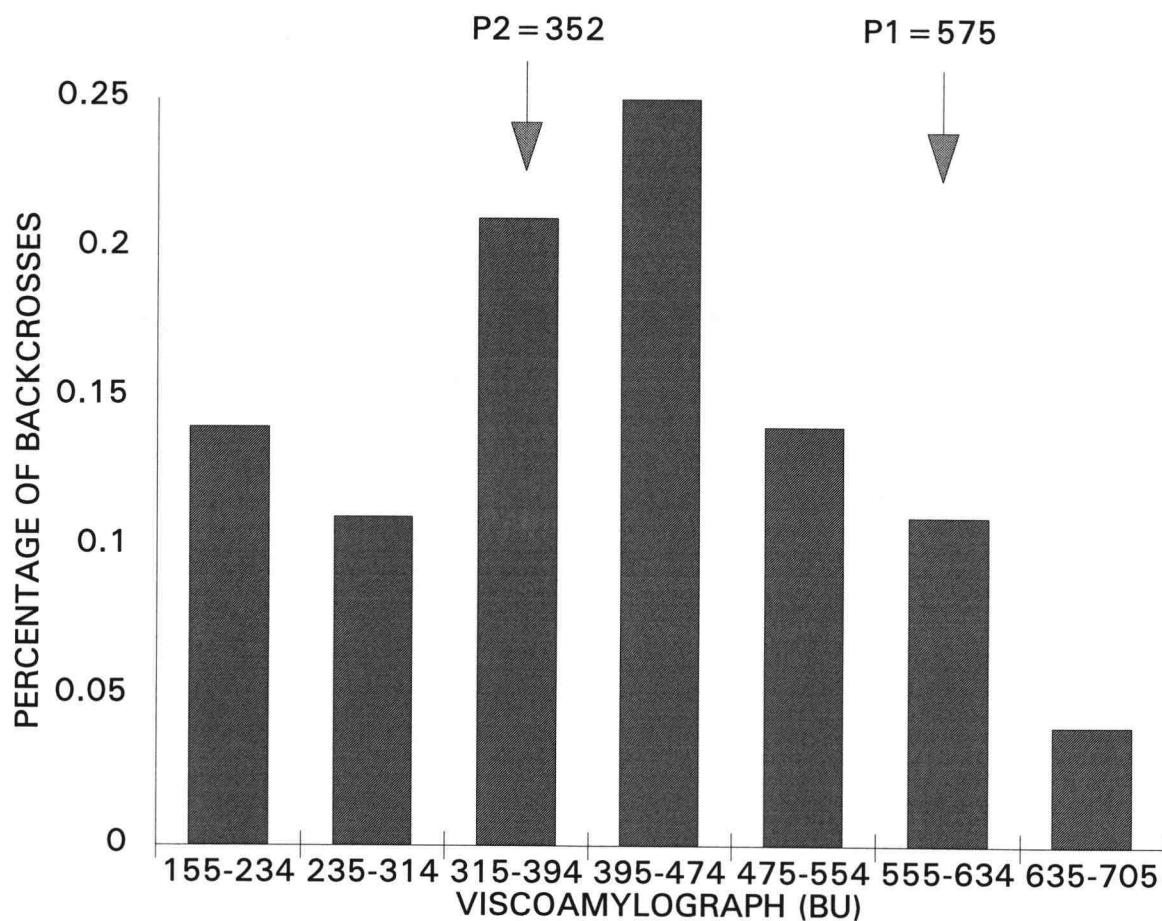


Figure 5. Frequency distributions of viscoamylograph values measured on reciprocal backcross populations ( $n = 28$ ) and mean values of OR#880485 (P1) and OR#890214 (P2) obtained in 1995.

this histogram is the skewed distribution toward lower values. Very few individual backcrosses exceeded the level of the highest parent (parent 1). Figure 6 shows a more normal distribution of backcrosses for the flour swelling volume test with individuals being observed both above the highest parent (parent 1) and below the lowest parent (parent 2).

Frequency distributions of the remaining three traits measured are given in Appendix Figures 1, 2, and 3.

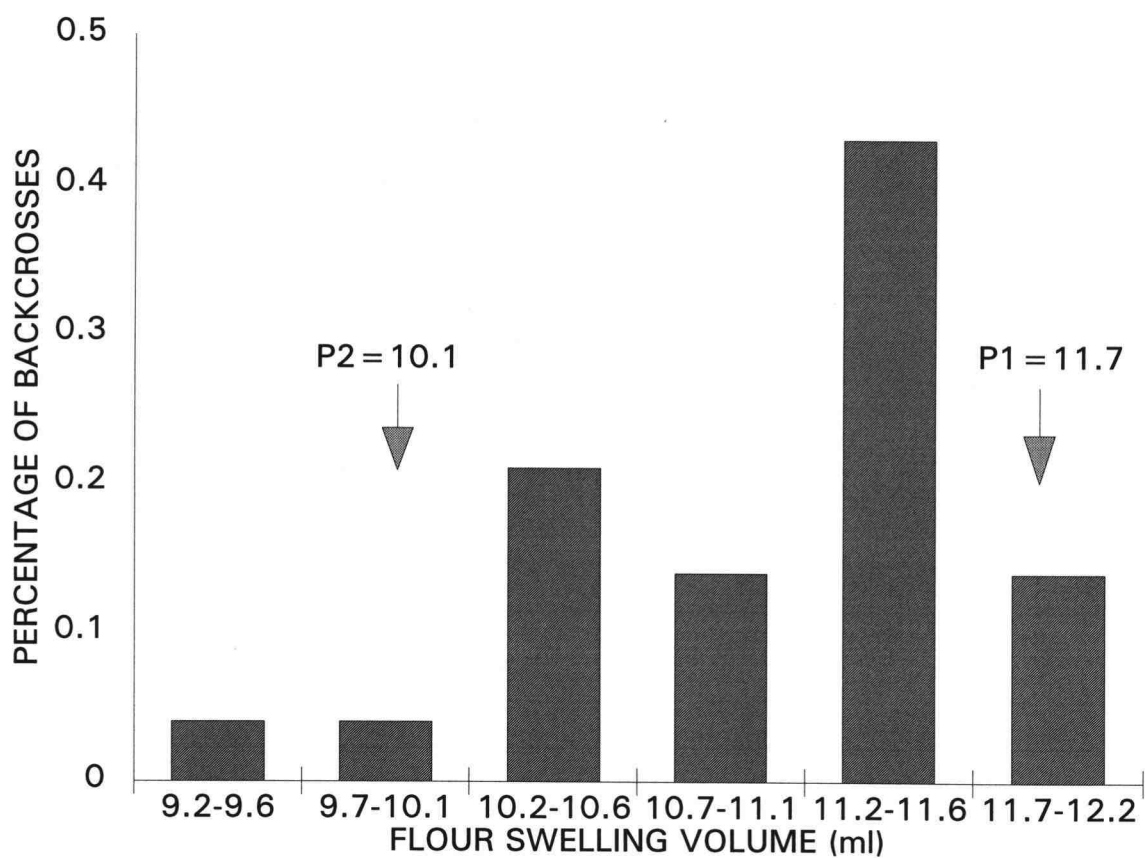


Figure 6. Frequency distributions of flour swelling volume values measured on reciprocal backcross populations ( $n = 28$ ) and mean values of OR#880485 (P1) and OR#890214 (P2) obtained in 1995.

## DISCUSSION

Noodle production is an important end-use of wheat flour in many Asian countries. Starch has been identified as the most important factor in flour for determining the overall quality of noodles (Kim and Seib, 1993 and Kruger, 1996).

The major objective of this study was to determine if the RVA or flour swelling volume procedures could substitute for the less suitable visco-amylograph. If so, they would be more appropriate to use in a breeding program, especially for early generation ( $F_2$  or  $F_3$ ) selection for noodle starch quality.

Parent, reciprocal  $F_2$ , and reciprocal backcross generations were space planted at the Hyslop Crop Science Field Laboratory to determine if associations existed between these procedures. In addition, information on grain protein and hardness and plant height was obtained. Differences in the experimental material were observed between and within generations for the viscoamylograph, flour swelling volume, and plant height. Coefficients of variation for flour swelling volume and plant height were low indicating a high level of precision whereas the coefficient of variation for the viscoamylograph was high, suggesting a low level of precision.

A possible year or environment impact on starch quality was noted. In 1994 parent 1 (OR#880485) had an RVA peak viscosity of 104. Parent 2

(OR#890214), the source of high starch quality, had an RVA peak viscosity of 151. In the previous year (1993) parent 1 had an RVA peak viscosity of 163 and parent 2 had an RVA peak viscosity of 283. Although the peak viscosities of these parents were much lower than in 1993, parent 2 had a higher value both years. An explanation for these lower levels of starch quality could be based upon the findings of Konik et al. (1993) that starch quality is dependent upon the growing season of the wheat.

In contrast to the 1993 and 1994 data, in 1995 parent 2 had a lower level of starch quality (150SNU) compared to parent 1 (167SNU). The most likely causes for this change are the low number of parental plants sampled and a possible year by genotype interaction.

A major limiting factor also is that the viscoamylograph requires 100g of flour according to AACC method 22-10 (1995). Unfortunately in this study it was necessary to reduce the flour sample to 45g per plant. Even with this reduction, 96% of the individual plant samples were eliminated due to insufficient sample size which left 45 samples for testing. Out of these 45 samples, two were from parent 1 and four were from parent 2. This led to high variances for the parental populations and questionable values for the means of each parent. Further studies involving the amylograph must be done in later generations when more seed is available and the lines are more genetically uniform.

Even with a large environmental effect the nature and frequency distributions from the RVA, viscoamylograph, and flour swelling volume suggests that there is genetic variability for starch quality within the populations employed in this study. However, with such a limited segregating population size it is difficult to speculate on the type of gene action involved.

The positive association between the RVA and viscoamylograph procedures agrees with previous studies (Panozzo and McCormick, 1993, Konik and Moss, 1993, and McCormick et al., 1991). The significant association between these two procedures ensures that both procedures are acceptable for measuring starch quality in wheat.

Crosbie et al. (1991) showed in a previous study that a highly significant positive relationship existed between the viscoamylograph peak viscosity and flour swelling volume. In this study a significant relationship was not detected between these variables.

Conflicting information also exists between the results of this experiment and previous unpublished data generated from the Oregon State University (OSU) breeding program. This study indicates that the relationship between the RVA and flour swelling volume is non-significant. However, in another study using 40 samples involving fixed lines from the OSU wheat breeding program, a highly significant ( $p < 0.001$ ) positive relationship between the RVA and flour swelling volume ( $r = 0.82$ ) procedures was found.

Perhaps larger sample numbers and other experimental populations are required to resolve these inconsistencies and achieve reliable results.

Moss (1980) found a positive relationship between the starch paste peak viscosity, as measured on the viscoamylograph, and protein content. The relationship between these two measurements was non-significant in this study. A negative relationship involving flour swelling volume and grain protein was found. The inconsistent relationship between starch quality and grain protein could be related to findings by Oh et al. (1985d) where the quality of the protein, not the quantity, is related to noodle quality. Previous studies have shown a positive relationship between starch quality and noodle quality (Nemeth et al., 1994 and Kim and Seib, 1993). This could mean that inconsistent relationships might be found between starch quality and protein content but a stable relationship between starch quality and protein quality could be found.

A negative relationship between grain hardness and flour swelling volume also was observed. This relationship is consistent with comments by several authors (Crosbie et al., 1990, Oh et al., 1985a, and Nagao, 1996) that soft wheats with a high level of starch quality are preferred for the production of noodles. Rho et al. (1988) also comments that some hard wheats can make good oriental noodles but in general, hard wheats make poor Japanese noodles.



Significant correlations between plant height and other procedures or traits were not found. This indicates that breeding for starch quality, grain protein, or grain hardness should be possible while maintaining desirable height levels.

## SUMMARY AND CONCLUSIONS

Parents,  $F_2$ , and backcross generations from the reciprocal cross involving winter wheat selections OR#880485 and OR#890214 were space-planted at the Hyslop Crop Science Field Laboratory. The major objective of this study was to determine the level of association between three procedures which give an indication of the overall noodle quality.

The following procedures and traits were measured on an individual plant basis: rapid visco-analyser, viscoamylograph, flour swelling volume, grain protein, grain hardness, and plant height.

An analysis of variance was calculated for each procedure and trait to determine if differences existed between the generations. Least square means were calculated for each generation. Fisher's protected LSD was used to identify significant differences between the mean values for each procedure and trait. The level of association between each procedure and trait was determined using phenotypic correlations.

The following conclusions were made based upon the results of this study.

1. Starch quality varied in both parents in the 3 years of the study. This suggests that a genotype by environment interaction exists for this trait and that both parental genotypes were influenced by the environment, however, not to the same degree.

2. Maternal effects were not observed as no reciprocal differences for these procedures and traits were found.
3. Analysis of variance indicated that significant differences existed among the generations for the viscoamylograph, flour swelling volume and plant height.
4. A significant positive association was found between the RVA and viscoamylograph procedures. This suggests that either of these two procedures can be used to test noodle starch quality in wheat.
5. The apparent genetic variability observed for starch quality from the RVA, viscoamylograph and flour swelling volume indicate that starch quality could be improved through breeding with the populations studied.
6. The significant negative association between grain hardness and flour swelling volume indicates that wheat can be developed with both a desirably soft grain and a high level of starch quality.
7. The viscoamylograph procedure is not suitable for early generation selection. This procedure requires too large a sample of flour to test in early generations or on individual plants, especially as in this study when the other tests require the same wheat flour.
8. Further experiments involving the flour swelling volume procedure are required to gain consistent results and reliable associations between this procedure and the viscoamylograph and RVA procedures for measuring noodle starch quality.

9. Experiments involving starch quality procedures and protein quality tests, such as SDS-sedimentation and SDS-page for wheat glutenins, might be helpful in determining the association between starch and protein. This association might provide another indicator of overall noodle quality.

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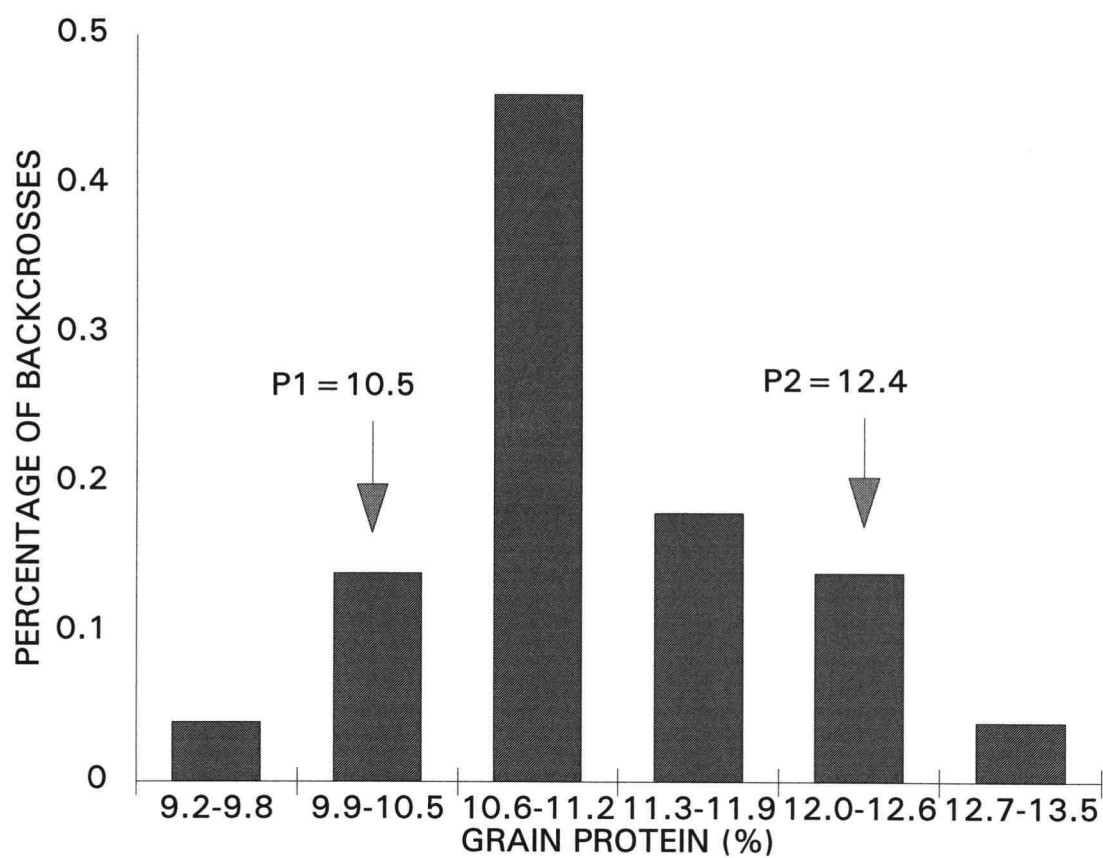
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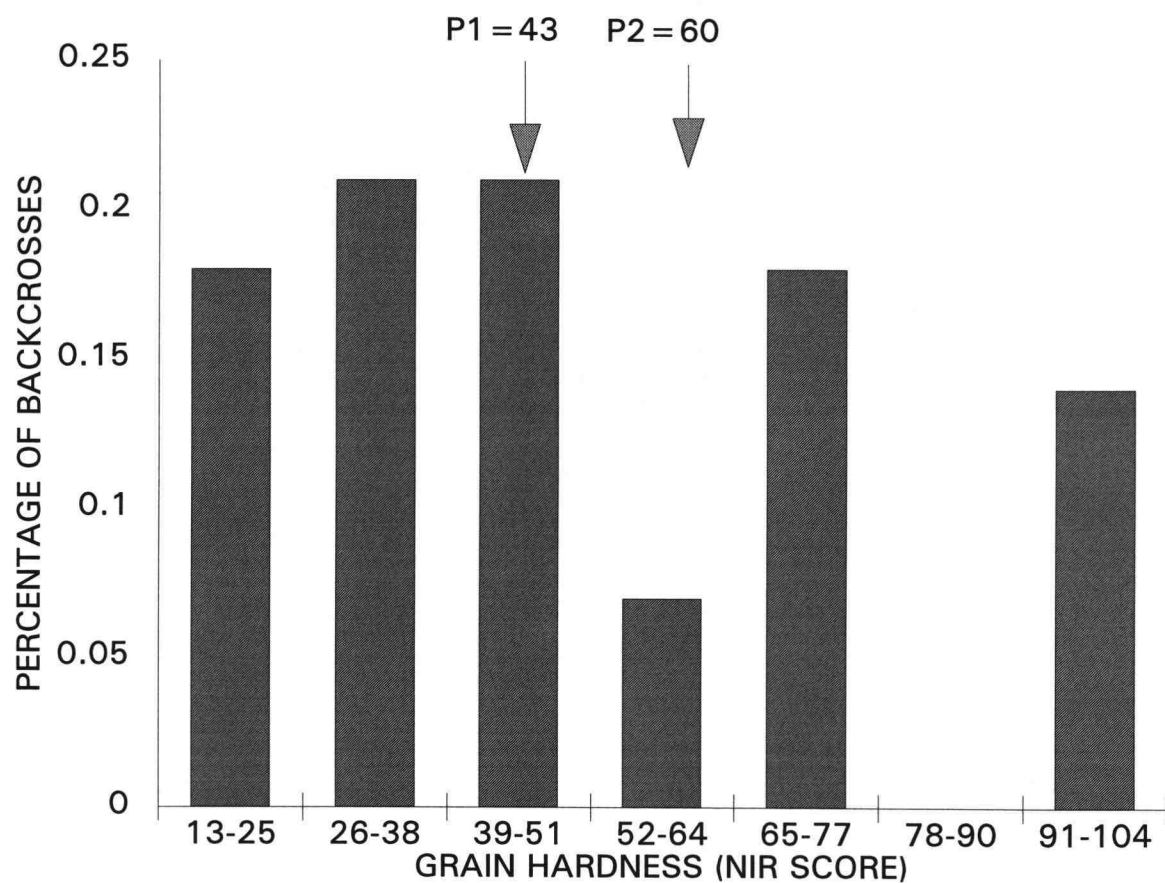
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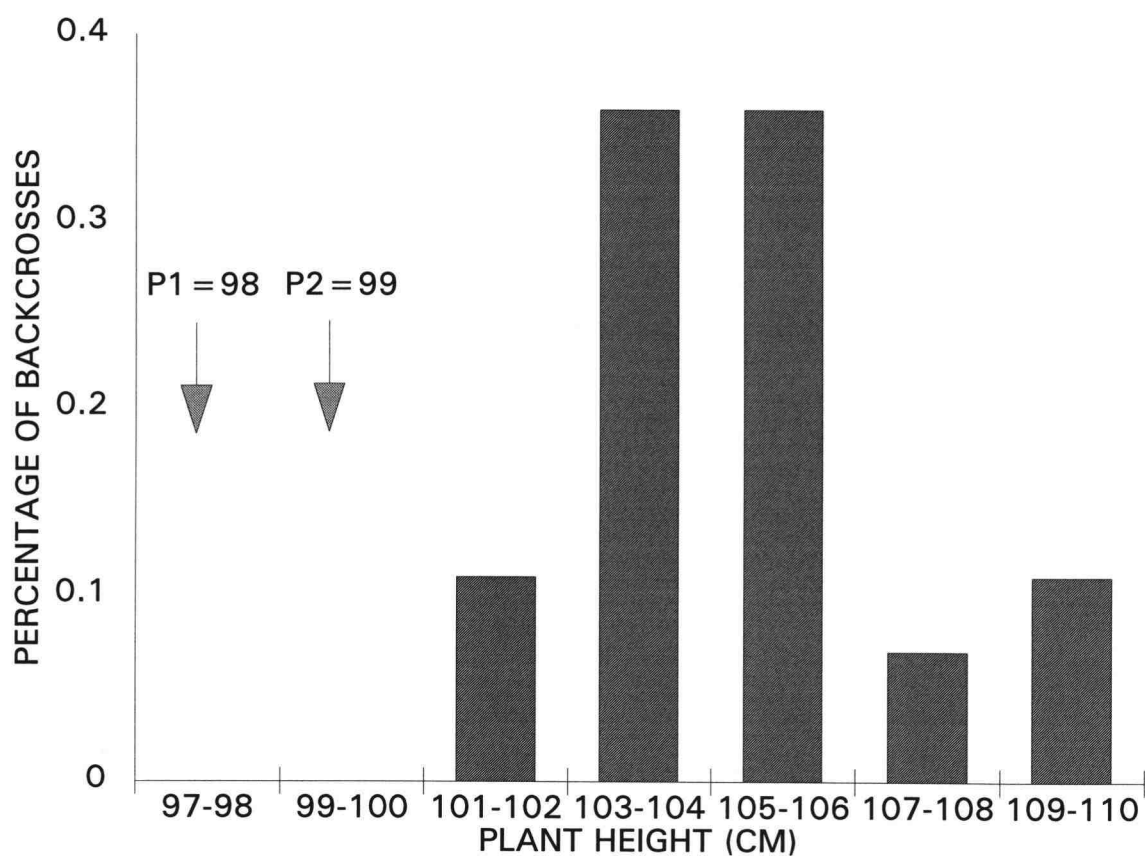
## APPENDIX



Appendix Figure 1. Frequency distributions of grain protein measured on reciprocal backcross populations ( $n=28$ ) and mean values of OR#880485 (P1) and OR#890214 (P2) obtained in 1995.



Appendix Figure 2. Frequency distributions of grain hardness measured on reciprocal backcross populations ( $n = 28$ ) and mean values of OR#880485 (P1) and OR#890214 (P2) obtained in 1995.

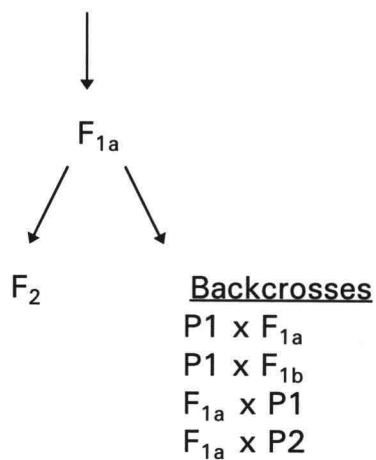


Appendix Figure 3. Frequency distributions of plant height values measured on reciprocal backcross populations ( $n = 28$ ) and mean values of OR#880485 (P1) and OR#890214 (P2) obtained in 1995.

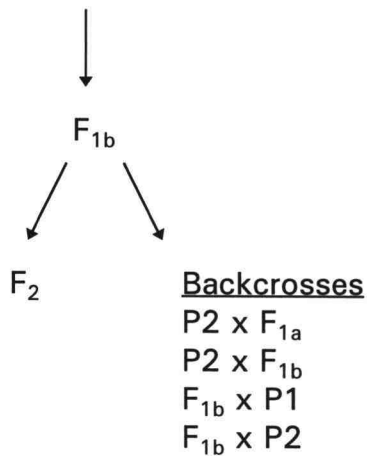
Appendix Table 1. A flow diagram of the generations used in this study originating from OR#880485 (parent 1) and OR#890214 (parent 2).

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Parent 1 x Parent 2



Parent 2 x Parent 1



Appendix Table 2. Means and standard deviations for the parents, reciprocal  $F_2$ , and reciprocal backcross generations for the Rapid Visco-analyser, Viscoamylograph, Flour swelling volume, Grain protein, Grain hardness, and Plant height obtained in 1995.

	RVA	Visco-amylograph	Flour swelling volume	Grain protein	Grain hardness	Plant height
Generation	(SNU)	(BU)	(ml)	(%)	(NIR Score)	(cm)
OR#880485	162.25 (13.40) *	575.00 (63.90)	11.68 (0.42)	10.45 (0.37)	43.48 (18.00)	98.25 (1.89)
OR#890214	152.00 (4.24)	352.50 (130.81)	10.11 (1.17)	12.35 (1.20)	59.80 (46.24)	99.00 (2.83)
$F_{2A}^1$	162.00 (14.98)	326.67 (83.35)	11.05 (0.34)	11.40 (1.24)	37.88 (23.92)	107.83 (5.19)
$F_{2B}^2$	139.80 (18.13)	279.00 (118.08)	10.68 (0.36)	10.68 (0.47)	64.96 (28.52)	110.80 (3.56)
BC/OR#880485	158.18 (26.40)	391.53 (162.30)	11.17 (0.48)	11.01 (0.95)	46.32 (19.51)	105.47 (2.72)
BC/OR#890214	161.91 (15.97)	408.36 (79.01)	10.91 (0.94)	11.34 (0.92)	59.28 (35.03)	104.00 (1.90)

\* Standard deviation in parentheses

<sup>1</sup>  $F_2$  populations originating from the cross OR#880485/OR#890214

<sup>2</sup>  $F_2$  populations originating from the cross OR#890214/OR#880485



Appendix Table 3. A summary of the climatic data at the Hyslop Crop Science Field Laboratory near Corvallis, OR. during the 1994-1995 growing season.

Month	Average Temperature (°C)			Precipitation	Evaporation
	Maximum	Minimum	Mean	(mm)	(mm)
October	17.8	4.4	9.2	97.5	63.5
November	8.8	0.9	6.2	229.4	0.0
December	8.9	2.1	4.5	159.0	0.0
January	9.0	3.5	3.2	251.5	0.0
February	12.5	3.5	5.6	108.9	0.0
March	13.6	3.0	7.4	120.4	0.0
April	14.9	3.9	12.5	134.6	70.1
May	20.9	7.5	13.6	46.5	135.6
June	21.5	10.1	15.8	59.9	146.6
July	26.6	12.9	19.5	13.2	192.0

Appendix Table 4. Raw data of six procedures and traits measured in 1995.

Treatment <sup>1</sup>	Replication 1					
	RVA	Amylo-graph	Flour Swelling Volume	Grain Protein	Grain Hardness	Plant Height
1	147.0	480.0	11.6	10.1	69.3	98.0
1	158.0	615.0	12.3	10.2	33.4	101.0
3	144.0	175.0	10.6	10.7	79.7	105.0
4	152.0	445.0	10.6	11.0	99.2	114.0
5	168.0	370.0	11.3	9.2	48.6	101.0
5	159.0	361.0	11.3	9.9	58.6	103.0
5	132.0	385.0	10.9	11.7	51.4	103.0
5	136.0	218.0	11.3	10.9	43.5	105.0
5	166.0	600.0	10.9	11.1	42.3	110.0
5	121.0	217.0	10.6	12.5	71.1	110.0
5	170.0	565.0	10.9	12.5	71.3	110.0
6	139.0	315.0	10.6	11.4	103.6	103.0
6	160.0	412.0	10.9	10.6	69.8	103.0
6	153.0	305.0	10.3	11.1	100.0	106.0
6	150.0	395.0	9.3	13.5	101.3	106.0
6	161.0	410.0	9.9	11.8	95.4	106.0

Treatment <sup>1</sup>	Replication 2					
	RVA	Amylo-graph	Flour Swelling Volume	Grain Protein	Grain Hardness	Plant Height
1	165.0	595.0	11.6	10.6	29.3	97.0
1	179.0	610.0	11.3	10.9	57.9	107.0
2	155.0	445.0	9.3	13.2	92.5	97.0
3	188.0	315.0	11.6	10.4	12.8	111.0
3	162.0	325.0	11.3	10.9	25.2	104.0
3	153.0	375.0	10.9	11.9	32.7	116.0
3	158.0	350.0	10.9	10.8	50.6	102.0
4	121.0	115.0	10.3	10.9	57.9	107.0
4	120.0	250.0	10.6	10.6	58.3	115.0
4	147.0	285.0	11.3	11.0	24.9	110.0
5	182.0	510.0	10.3	11.3	68.8	105.0
5	176.0	430.0	11.3	10.6	63.5	105.0
5	181.0	705.0	11.3	12.3	46.0	104.0
5	91.0	230.0	11.3	10.0	39.9	104.0
5	180.0	550.0	11.9	10.8	20.1	108.0
6	178.0	515.0	12.3	10.4	15.9	105.0
6	177.0	565.0	11.6	10.8	22.9	105.0
6	160.0	420.0	10.3	12.4	36.4	105.0

Appendix Table 4. (continued)

Replication 3						
Treatment	RVA	Amylo-graph	Flour Swelling Volume	Grain Protein	Grain Hardness	Plant Height
3	167.0	420.0	10.9	13.7	26.3	109.0
5	185.0	530.0	10.3	10.6	71.3	105.0
6	196.0	395.0	11.3	10.8	38.9	101.0
6	152.0	330.0	11.3	11.2	29.3	101.0
6	155.0	430.0	12.3	10.7	38.6	103.0

Replication 4						
2	149.0	260.0	10.9	11.5	27.1	101.0
4	159.0	300.0	10.6	9.9	84.5	108.0
5	188.0	340.0	11.6	10.0	27.7	104.0
5	149.0	255.0	11.6	11.9	16.1	104.0
5	161.0	235.0	11.9	11.1	13.6	104.0
5	144.0	155.0	11.3	10.7	33.6	108.0

<sup>1</sup> Treatments 1-6 correspond to generations 1-6 (P1, P2, F2-P1xP2, F2-P2xP1, BC/P1, and BC/P2 respectively).