

Genotype-by-Environment Influence on the Traits Associated with Natto  
Quality

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## Intro

Soybeans are primarily grown for their high protein and oil content that make them an important crop for oil manufacturing, animal feed, and food production (Roa et al., 2002; Wilcox & Shibles, 2001; Hymowitz et al., 1972). The estimated soybean production for the U.S. in 2004-2005 was 85.5 million metric tons (C&A Report, 2005) estimated at a value of over \$16 billion (USDA, 2005). The success of the soybean industry is attributed to the crops composition (Liu, 1997). Roughly 60% of a soybeans dry weight is composed of protein and oil making it number one for protein content and number two for oil content among other cereal and legume species (Liu, 1997).

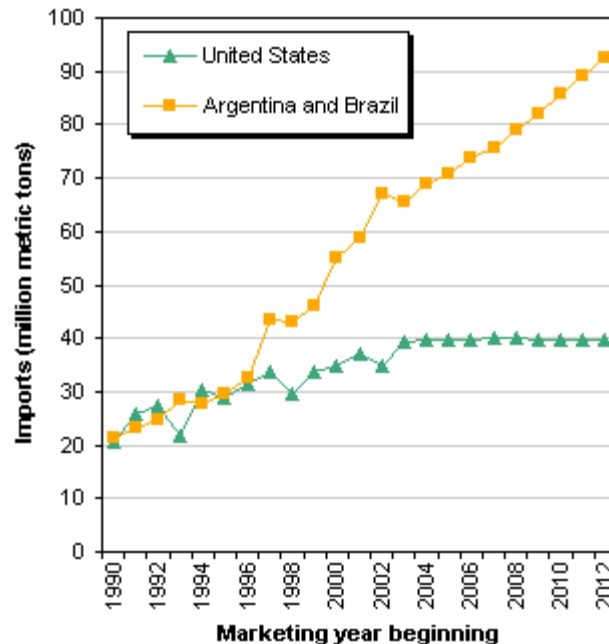
Not all soybean cultivars are the same, however, and a great deal of variation exist for seed composition and agronomic value. Some variations are due to environmental factors while others are genetically controlled and lead to different cultivars suitable for various products. The variations in soybeans have led to a variety of different soy based products worldwide. The different uses of soybeans have risen from two distinct cultures; East and West (Liu, 1997). Far East countries have used soybeans to make tofu, natto, miso, tempeh, and bean sprouts prepared for direct human consumption. These are also called food beans. In western cultures, soybeans are often ground and processed into defatted meal and soybean oil products. Animal feed is the main use for defatted soy meal, while soybean oil is processed and used in various food products such as cooking oils, soy flour, soy yogurt and cheese, and soy meat analogs (Liu, 1997).

Soybean cultivars used as food beans are much more specific than those used to make meal or oil products because they must meet specific quality standards. Appearance and seed composition are much more important for raw bean products. Also, multiple cultivars must be developed between food bean products because desirable traits for one product are not necessarily the same for another. An example is the difference between soybeans used to prepare natto and tofu. Natto soybeans must be small and soft with little attention to protein during selection, while tofu soybeans are larger and are selected for high protein content (Liu, 1997). Plant breeders interested in developing food grade soybeans must identify superior cultivars that meet the specific requirements.

International soybean trade is mainly for meal and oil products while food bean products occupy a small percent of the market (Liu, 1997). Although food grade beans currently represent a small part of the international soybean market there is confidence that the market will continue to grow. One reason for this optimism is that foods containing soy products are reported to offer health benefits. Protection against certain types of cancer, one of the leading causes of death in developed countries, is one major proposed health benefit (Liu, 1997; Messina, 1999). Certain proteins, lunasin and lectin, isoflavonoids (McCue, 2004; Sliva, 2005), and other phytochemicals (Singh & Agarwal, 2005) found in soybeans have been reported to offer novel protection against cancer. Liu (1997) also reviews a variety of research dealing with the possible health benefits of soybeans.

The production of food grade soybeans may be important for U.S. soybean export. The United States share of the international soybean market has been declining due to more intense soybean production in South America (Durham, 2003). Figure one illustrates the increasing soybean production from Argentina and Brazil.

Figure One



(Durham, 2003)

This graph shows that Argentina and Brazil now **export** more metric tons than the U. S. and the two countries exports are projected to grow. As mentioned above, food grade beans must meet very specific quality standards. Since the U.S. cannot out-produce other nations it may be economically important to produce high quality soybeans used in this small market. The practice of quality not quantity may be important.

The soybeans imported by Asian countries that supply these small markets are used to prepare a variety of foods. Japan imports food grade soybeans for a product named natto; a traditional Japanese food prepared by soaking, steaming, and fermenting whole soybean seeds (Cober et al., 2001). Natto is an important component of the Japanese diet and a high quality product is essential. Soybean cultivars are selected for natto production based on a variety of criteria. The quality of natto can be affected by a variety of variables including soybean cultivars used in manufacturing, alterations in the preparation, and the bacterial strain used to carry out fermentation (Wei, 2004). Niche food bean products such as natto may provide the United States a new soybean market through development of superior cultivars that meet the quality standards needed for natto production.

Seed size and appearance influence the acceptability of a soybean for natto production (Cober et al., 1997; Taira et al., 1987). A seed size range of 5-5.5 mm is acceptable, and generally for natto production the smaller the better (Wilson, 1995; Griffis & Wiedermann, 1991). Small soybeans are desirable because they have high water absorbing capacity, high carbohydrate content, and low oil (Wei & Chang, 2004). The appearance of soybeans for natto production is also important. Soybeans with a

small, uniform size and smooth, light colored coat are desirable for natto production (Wei & Chang, 2004).

Other Important factors determining cultivar suitability for natto production are carbohydrate content of raw seed, water weight increase ratio (WIR), cracking ratio of steamed soybeans, firmness after steaming, and ammonia content (Wilson, 1995; Geater et al., 2000; Wei & Chang, 2004). These traits are controlled by a variety of chemical components that interact and can be influenced by environmental conditions during seed development. In a breeding program chemical components of seeds are important because they are genetically determined which makes it possible to select for them in a breeding strategy. There has been a great deal of research to find correlations between chemical components of soybeans and desired physical properties. A correlation between the two gives a breeder a more reliable character to select for.

One important chemical component of soybean seeds is carbohydrate composition, which is on average 35% of a soybean seed on a dry matter basis (Liu, 1997). Carbohydrates are comprised of simple sugars, oligosaccharides and polysaccharides. Carbohydrates are also broken down into water soluble and insoluble components.

Carbohydrate composition has been correlated to a number of physical properties of natto, two of which are water absorption and seed hardness (Taira et al., 1983; Geater et al., 2000). Water absorption is an important characteristic for natto production because it directly affects seed hardness (Tiara, 1990) which affects the palatability of natto and its production. Taira et al. (1983) reported that high carbohydrate content is correlated to a high water-absorbing capacity. Geater et al. (2000) also reports that increased total sugar and its components were associated with increased water absorption, water loss, and decreased natto hardness. High water absorption and low steamed hardness are important for consumers who desire soft steamed seeds (Cober et al., 2001; Tiara, 1990). Manufacturers are concerned with this because the time required for seeds to absorb sufficient water directly affects production costs. "Stone Seeds" defined as seeds that uptake absolutely no water (Cober et al., 2001) are detrimental to manufacturers due to wasted time and money. It has been reported that hard seeds are often a problem for North American soybean cultivars (Saio, 1976). Taira et al. (1987) concluded that Japanese cultivars are softer than American cultivars because they have a higher weight increase ratio (WIR) after soaking. North American soybean breeders developing natto cultivars need to over-come this issue. These results indicate a positive relationship between carbohydrate level and water absorption, a negative relationship between carbohydrate level and seed hardness, and a negative correlation between water absorption and seed hardness.

Natto taste is carbohydrate dependant. High carbohydrate content in soybeans has been negatively associated with ammonia content (Wei & Chang, 2004; Ohta, 1986) which is associated with hard seeds and undesirable smelling natto (Taira, 1990). High carbohydrate content is also positively correlated with desirable natto viscosity and stickiness (Wei & Chang, 2004). These sensory qualities are important for consumers who desire a consistent product and the problem can be addressed through breeding.

Carbohydrate composition is also important because the fermentation process for preparing natto is dependant upon sugar levels. Taira (1990) showed that high sucrose levels can cause a spike in temperature that inhibits microbial productivity during the

initial stages of fermentation. Stachyose is a more suitable energy source than sucrose because it is broken down gradually and serves as a more stable energy source throughout the fermentation process, giving an even temperature which is desirable (Wei & Chang, 2004; Taira, 1990). Taira et al. (1989) conducted research on 105 soybean lines in Japan and reported mean values on a dry weight basis for total free sugar (11.5%), sucrose (6.4%), stachyose (3.31%), and raffinose (0.81%). These values can be used to evaluate soybean lines for potential natto production.

While this information is valuable to breeders trying to select for specific chemical components, developmental variance can make breeding for these traits difficult. Environmental conditions can influence the development of the seed and affect its composition. In parts of the U.S., Europe, and China protein levels in soybeans have shown to be lower in the northern latitudes due to lower temperatures and higher rainfall (Vollman et al., 2000). Kumar (2006) reported a significant negative correlation between protein and latitude, and a positive correlation between oil and latitude at  $P < 0.01$ , and  $P < 0.05$ , respectively. Vollman et al. (2000) showed that during years of low precipitation and high temperature, protein synthesis was higher than oil synthesis in soybean seeds. The timing of rainfall and temperature with respect to plant maturity is also important (Foroud et al., 1993; Wolf 1982). Foroud et al. (1993) reported that the level of soil moisture most affected protein, while the timing with respect to maturity of irrigation controlled oil content. During the seed fill period high amounts of precipitation can lead to higher oil content in soybeans (Vollman 2000). Sucrose and stachyose levels were also affected by the environment. They were both reduced in soybeans as temperature was increased, with sucrose showing the steepest level of decline per degree increase (Wolf et al., 1982).

These interactions can pose difficulties for plant breeders. A plant's development or phenotype is determined by its genotype, the environment, and the interaction between the two. The environmental components of development, controlled by biotic and abiotic factors, are usually of little concern to plant breeders for variety selections. Plant breeders can make cultivar selections based solely on genotypic effects when genotype by environment interaction do not exist, which is often not the case (Kang, 1998). An interaction between the environment and a genotype is called genotype by environment interaction (GxE). It can be seen when cultivars are grown in different locations and the genotypic responses are different between locations and one cultivar is superior in one environment but not the other (Kang, 1998). This is the most confounding GxE interaction, referred to as cross-over effects, where a component such as mean yield for cultivars changes ranks across environments. Rank changes for a character caused by GxE interactions can make cultivar selection difficult for plant breeders (Kang, 1998). For a plant breeder to make progress developing a crop he or she must understand if the desired character has genotype by environment interaction.

Estimating and understanding these interactions can be very difficult due to large interrelated data sets. It is important to understand these interactions, however, for two reasons. One is that a large GxE interaction for a trait can lead to crop instability already discussed as a cross-over effect. This could have profound effects at a national level if a cultivar is recommended based on trials in one environment and then grown in another environment that is unsuitable for similar development, a concept discussed by Kang (1993). The other reason GxE interactions are important is that they may offer some

opportunities for exploitation of the environment (Kang, 1998; Annicchiarico 2002). If GxE is well characterized, it is possible to develop locally adapted cultivars with high, consistent performance.

Based on results from field trials, Tiara (1990) indicated that cultivars rather than their environments primarily control the qualities of soybeans. Data from a different trial agreed with this conclusion (Geater et al., 2000). This is not to say, however, that cultivars will always perform the same across environments. Cultivars with specific environmental adaptations will not perform the same in different environments. Geater et al. (2000) concluded that the importance of genotype and environment would be controlled by the variety of the genotypes and environments considered. These results indicate that a breeding program must evaluate different cultivars at locations that represent their target production environment.

The other factor to address for cultivar development is a traits heritability. A plant breeder may waste time and resources trying to select for a trait that is not heritable. Plant breeders make selections based on phenotypic traits that are controlled by genetic and environmental components. For selection to be effective, the trait must be mainly controlled by genetic factors that can be controlled and influenced by selection. There are two types of heritability that researchers recognize; broad and narrow sense heritability (Falconer & Mackay, 1996). Heritability in the broad sense is the amount to which a character is controlled by genetics (Falconer & Mackay, 1996). A multi-environment trial can be used to address this issue. This allows a researcher to partition out how much a character is being influenced by its genotype, the environment and the extent of the GxE interaction (Falconer & Mackay, 1996). The less GxE interaction on the character, the more it is controlled by the plants genetic make up and the more control a plant breeder has to improve the trait.

Just because the character is controlled genetically, however, it might not be passed from parent to off-spring. If a breeder has a plant with a desirable phenotype that he or she would like to pass on the trait be controlled by narrow sense heritability. Narrow sense heritability is the extent to which a trait can be passed from generation to generation by genes, also referred to as resemblance between relatives (Falconer & Mackay 1996). There are a variety of experiments that can determine a traits heritability in the narrow sense. The higher the narrow sense heritability the more likely a character will be passed from parent to off-spring. A cultivar with high heritability for desired traits is preferred. The reason for this is that characters with high heritability can be improved more quickly (Nyquist, 1991).

Panthee et al. (2005) reported that 54-71% of the variation for protein, oil, and seed size in his population were attributed to genetic variation. High heritabilities for protein (0.89) and oil (0.84) were reported by another group of researchers (Chung et al., 2003). Water absorption is also a genetically controlled trait, so selection in a breeding program can have an impact, but it must be evaluated across environments due to environmental influence during seed development (Geater et al., 2000; Cober et al., 2001). Seed size, another important natto quality, has been evaluated for heritability. One group of researchers reported heritability estimates from 0.44 to 0.94 (Burton, 1987) while another groups realized heritability estimates ranged from 0.26 to 0.50 (Cober et al., 1997). The discrepancy between these data could be the result of different populations, environments, or different methods of evaluation. Both data sets indicate,

however, that seed size is a heritable trait and plant breeders can use it as a selection criterion. Continued research into the reliability and heritability of soybean cultivars for natto production may lead to an economically important soybean cultivar for breeding programs.

### Objective

The objective of my research is to identify quality traits associated with desirable natto cultivars that have less genotype by environment interaction. Traits with a lot of GxE interaction are difficult for plant breeders to improve and superior cultivars are hard to select (Kang, 1998). A trait must be predominantly controlled by genes for it to be manipulated, and the larger a GxE component for a phenotypic character the less progress through selection can be made.

It is important for a plant breeder to be aware of this so he or she does not waste resources trying to select for an ambiguous trait. When there is significant GxE interaction, a superior cultivar in one environment may not perform similarly in another, known as a cross over effect (Kang, 1998; Annicchiarico, 2002). Correlations among phenotypes and genotypes are not possible when these types of confounding GxE interactions are present (Kang, 1998). I expect to identify one or more traits that are controlled more by a plant's genes than by GxE interaction.

### Importance

The objective of this project is to help gather knowledge that will lead to improved natto soybean quality. This is very important for both farmers and producers. As mentioned above, natto production is very specific and soybean quality used to make natto is the most important factor. If a farmer does not have a reliable soybean cultivar that he or she knows will meet the quality standards they may have an economic loss. Producers prefer to purchase a consistent product so they can maintain production practices and working relationships with the same growers.

The importance can also be seen on an international level. As other countries out produce the U.S. in soybeans it will be important to maintain a high quality soybean to ensure continued agronomic profit. The U.S. does not have the resources to out yield other countries (Durham, 2003) so niche markets that demand high quality beans may provide another outlet for the sale of American crops. This research will help identify the natto quality traits that a breeder will have the most success improving and help identify the cultivars with the most reliable performance. This information will directly benefit both growers and producers of natto soybeans.

### Materials and Methods

To identify which natto quality traits have less GxE interaction I will conduct a multi-environment trial. It will consist of twelve soybean cultivars (MFS-591, MFS-511, SS-516, V00-3493, V00-3488, V00-3636, V01-4937, Teejay, Camp, Hutcheson, 11, 12) grown for two years in six environments over five locations (Blacksburg, Warsaw,

Suffolk, King Williams, Mt. Holly). I will use a randomized complete block design (RCBD) with three replications per site.

20x10 foot plots with 4 rows, 30" apart will be planted in Blacksburg under full season conditions. Only the inner two rows will be harvested to avoid inter-genotypic competition.

In Suffolk the field plots will be planted using a Hege cone planter in a double crop system after barley. The soil type is Enola loamy fine sand. The plots will be 17x24 feet with 5 rows 15" apart. The inner three rows will be harvested to avoid competition. The seeding rate will be 800 seeds per plot.

In Warsaw both full season and double crop soybeans will be grown. The full season plots will be 18x10 feet with 4 rows. They will be planted with an Almaco cone planter with 30" between rows and only the inner two rows will be harvested. The double crop plots will be planted after barley with a Great Plains no-till drill with a Hege cone. The plots will be 5 rows planted 7.5" apart and 18 feet long. All rows will be harvested.

Field trials will also be conducted in King William county. The plots will be 20x10 feet with 4 rows, 30" apart. They will be planted in a FS/DC under irrigation. The inner two rows will be harvested.

Mt. Holly will have another set of experiments in a double crop system after barley under irrigation. The rest is yet to be determined.

A variety of phenotypic data will be collected to help identify which natto traits are less influenced by GxE. Both physical and chemical properties will be tested. Seed size, water absorption, seed coat strength, and steamed hardness are physical properties that will be tested.

Seed size will be estimated using a weight to soybean number ratio. One hundred soybean seeds will be randomly selected and weighed. These size data will be reported as grams per hundred seed and will be used to evaluate soybean size.

The water weight increase ratio (WIR) will be tested using a method adapted from Taira et al. (1987) and Wei & Chang (2004). Twenty grams of soybeans will be measured out and soaked in 100ml of water for 16 hours at room temperature. The water will be drained off and the soybeans weighed again. The (WIR) will be expressed as the weight of the soaked beans divided by the weight of the original sample (Wei & Chang, 2004).

The soaked soybeans will then be steamed to test for seed hardness and broken bean ratio after steaming. The soaked samples will be steamed in an autoclave at 250 degrees F at a pressure of 15 p.s.i. for 20 min. The steamed beans will sit at room temperature for two hours to allow for cooling.

To determine the seed coat strength a broken bean ratio will be calculated using a method adapted from Wei et al. (2001). The steamed beans will be weighed out and recorded. Then the broken beans will be removed and the sample will be weighed. The broken bean ratio will be the weight of the broken beans divided by the original sample. These data will be used to evaluate the seed coat strength of the soybeans.

Seed hardness will be measured using an Instron Universal Testing Machine with a 5000N load cell. Forty grams of steamed beans will be weighed out and placed in a blade box. The samples will then be run using a program developed by (SOMEONE). Hardness will be determined by the peak load in newtons.

The chemical components that will be tested are carbohydrate, protein, and oil content. Carbohydrate content will be analyzed using HPLC. A protocol designed by Dr. Maroof's lab will be used to evaluate sucrose, stachyose, and raffinose content in raw soybean seeds.

Protein and oil content will be measured using Near Infrared Reflectance by a lab in Iowa.

Average daily temperature and rain fall will be collected for the different areas. Field observations will be made as needed for maturity and pathogen attack. These data will be used to help explain the results.

### Rationale

GxE multi-environment field trials have been conducted in a variety of well-documented studies (Cooper & Delacy, 1993; Cooper et al., 1995; Basford & Cooper, 1998; Annicchiarico, 2002; Lee et al., 2003; Ibanez et al., 2005; Dehghani et al., 2006). I have chosen to use RCBD with three replications. The plots will be replicated to allow for statistical analysis of the results. RCBD is appropriate for this experiment because there are a limited number of entries and only one variable tested; varieties. There are other designs available field tests but they are less flexible for entry size and more applicable for multi-variate trials (Leclerg et al., 1939).

Another rationale to use RCBD is that the data generated can be analyzed with a variety of methods. It is important to have this flexibility because there are many analytic techniques available for GxE interaction analysis (Cooper & Delacy, 1994; Basford & Cooper, 1998; Yan, 2001). No one technique is widely accepted as the best because they all serve a different purpose and should be used based on the researchers objectives (Basford & Cooper, 1998). Although no one technique has been widely accepted as the best, their relative importance has been shown. One researcher (Annicchiarico, 2002) showed that yield gains increased by 4-5% when recommendations were based on modeled yield response rather than observed yields. This indicates that a breeding program can make more improvement from year to year in cultivar development if they incorporate modeling analysis into their selection strategy.

### Expected Outcomes

Using phenotypic data collected at two sites for two years on 12 natto soybean cultivars, I expect to identify one or more traits with minimal GxE interaction that will allow for effective progress through selection. I expect cultivars to be a more significant factor for variation among traits than environmental factors (Tiara, 1990; Geater et al., 2000). By identifying the GxE component of certain natto quality traits resources of a breeding program can be more economically allocated. This will help a breeding program make quicker progress in cultivar development.

I also expect to identify cultivars that could serve as good parents for specific traits to be used in future crosses. Parent selection is important in a breeding program when a desired trait is identified. Parents with good agronomic traits must be identified before the trait can be moved into locally adapted varieties.

If significant GxE interactions do occur I hope to make some correlation between the environmental data collected and the experimental results. This information could be useful to help identify the main environmental components that affect natto quality traits. This may allow a grower to predict or exploit the quality of his or her soybean crop prior to harvest.

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