INHERITANCE OF SOLUBLE OLIGOSACCHARIDE CONTENT OF SOYBEAN SEEDS

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ABSTRACT

Sucrose, raffinose, and stachyose make up the majority of the carbohydrates in soybean seeds. While sucrose is a desirable component of soybean seeds, raffinose and stachyose are considered to be anutritive factors and eliminating or reducing them appears to be a beneficial endeavour. The major objective of this study was to determine the genetic mechanism controlling accumulation of soluble saccharides in soybean seeds. An experimental soybean line, V99-5089, with high sucrose (14.6%) content coupled with low amounts of raffinose (0.5%) and stachyose (0.4%) was the center of this study. Three populations were studied and segregation patterns were observed in $F_{2:3}$ populations.

All three sugars were extracted by an aqueous procedure and quantified by high pressure liquid chromatography (HPLC) using a NH_2 column and refractive index (RI) detector. Segregation of seeds from $F_{2:3}$ plants indicated a single, partially recessive gene reduced stachyose content of soybean seeds from about 4% to less than 1%. Estimates of genetic variability indicate the presence of sufficient additive variation in addition to the putative major gene to warrant selection.

Raffinose and stachyose were positively correlated to each other and each was negatively correlated with sucrose while there was not a significant correlation between total sugar content and the amount of any of the individual sugars. Agronomic traits evaluated do not appear to be adversely effected by the reduction of stachyose content. Additionally, a negative relationship was observed between inorganic phosphorus and stachyose content of soybean seeds but a relationship was not observed between stachyose and phytate phosphorus or between inorganic phosphorus and phytate phosphorus.

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INTRODUCTION

Soy foods are consumed by humans as supplements, additives, snacks, and main dishes because of their high protein. Likewise, soybean meal is a common supplement in many types of livestock feed.

While there are carbohydrates in all seeds, they comprise the bulk of some crop seeds like corn but are less evident in others like soybeans. Sugars and other carbohydrates are important to plants in regard to stress tolerance and also play an essential role in the products made from the grain of many plants. The three major sugars found in soybean seeds are sucrose, raffinose, and stachyose; these three sugars make up 99% of the sugars found in soybean seeds.

Consumption of soybean products often results in gastrointestinal (GI) irritation as well as increased levels of flatulence. These symptoms are generally attributed to the raffinose family of oligosaccharides (RFO), also known as raffinosaccharides, which include raffinose, stachyose, and more complex sugars. In the case of soybeans, raffinose and stachyose are the predominant raffinosaccharides present. Ingestion of these compounds results in GI irritation because humans do not possess the α-galactosidase and β-fructosidase enzymes to hydrolyze the bonds that link the sugar moieties. *E. coli* bacteria in the large intestine digest these sugars and create gaseous by-products which have the side effect of discomfort for many people. The increased flatulence attributable to raffinosaccharides is a major factor limiting human consumption of soybean products. A decrease in the amount of raffinosaccharides in soybean seed should make soybean products more amenable to human consumption.

In addition to the potential for increasing the digestibility of soybeans to humans, reducing the level of raffinosaccharides in the seeds would be beneficial to livestock as well. Low raffinosaccharide content meal results in improved feed efficiencies for poultry and livestock by allowing better utilization when consumed.

In recent years, the demand for "food type" soybeans has been increasing due to increasing awareness of the health benefits of soy food consumption. One current approach to creating "food type" soybeans is to change the proportions of sugars present in the seed with little effect on the total amount of sugar content. The ideal soybean seed

would have almost all of its sugar in the form of sucrose with only trace amounts, if any, of the raffinosaccharides. The first step on the journey to creating the ideal soybean seed is to understand the genetic mechanisms at work in the formation of sucrose, raffinose, and stachyose.

Increased awareness of the problems associated with raffinosaccharides, has prompted research on the biochemistry of raffinosaccharide formation, but the details of several pathways have not yet been resolved. On the biochemical level, *myo*-inositol serves as a link between the raffinosaccharides and phytic acid, another anutritive component of soybean seeds for humans and non-ruminant animals. Phytic acid has a net negative charge and readily complexes available cations making them unavailable for use in necessary biological functions. For this reason, phytic acid contributes to various deficiencies such as Mg, Ca, and Zn in addition to reducing phosphorus availablity for use by monogastric animals. This makes reducing the phytate content of grains a worthwhile objective. Researchers have observed a relationship between low raffinosaccharide content and low phytate phosphorus content in seeds and ideally, both of these components could be minimized in grain crop seeds.

An experimental line in the Virginia Tech soybean breeding program, V99-5089, was found to have above average amounts of sucrose and low raffinose and stachyose contents. The objective of this study was to determine the inheritance patterns of sucrose, raffinose, and stachyose content in soybean seeds. Three populations were used for the study, the first consisted of a re-creation of the cross from which V99-5089 was derived and the other two populations were derived from crosses between soybeans containing normal levels of saccharides and V99-5089. Selected materials were analyzed for inorganic and phytate phosphorus concentration to observe the relationship of these components in soybean seeds as well as their relationship to the soluble sugars.

LITERATURE REVIEW

Protein, oil, sugar, starch, organic acids, and amino acids account for 75% of the dry weight of typical soybean seeds (Yazdi-Samadi et al., 1977). Soluble sugar composition of developing soybean seed varies with cultivar (Lowell and Kuo, 1989) but sucrose has been reported as the predominant carbohydrate in soybean seeds (Kuo et al, 1988; Maughan et al., 2000). Raffinose family oligosaccharides (RFO) are present at relatively high levels in grain legume seeds (Jones et al., 1999) and Kuo et al. (1988) reported that all Leguminosae contain more stachyose than raffinose.

Soybeans are primarily grown for their oil and protein content (Hymowitz et al., 1972) but total sugar content of soybean cultivars is important to the soyfood industry (Geater and Fehr, 2000;Geater et al., 2001). One soy food product with high quality standards is natto; quality characteristics important in natto production include small seed, highly permeable seed coats, high water uptake, round seeds, high sucrose content, and low levels of Ca, oil, and raffinosaccharides (Cober et al., 1997). Geater et al. (2000) found protein+oil to be positively correlated with natto quality and other seed traits; this may be a useful selection criterion in breeding cultivars for the soy food industry.

Description of Raffinosaccharides

Raffinosacharide is a term that has been coined to refer to the raffinose family of oligosaccharides. There are several terms and acronyms used to refer to these compounds, some of them are: RFO (raffinose family oligosaccharides), RSO (raffinose series oligosaccharides), raffinose saccharides, and raffinosaccharides. These compounds are complex sugars formed by the addition of galactose residues to sucrose.

The first member of the RFO is raffinose which consists of sucrose with one galactose residue. Raffinose [α-D-galactopyranososyl-(1-6)-α-D-glucopyranosyl-(1-2)-β-D-fructofuranoside] is a tri-saccharide that has been detected in leaves, rhizomes, roots, seeds, and stems of numerous plant families. Stachyose [α-D-fructoruanoside] is a tetrasaccharide produced in the leaves of many plants. Verbascose results from the addition of a third galactose residue and more complex forms of raffinosaccharides are

created by the subsequent addition of galactose residues. In the case of soybean, the predominant raffinosaccharides are raffinose and stachyose.

Soybean meal is used primarily as a livestock feed (Wilcox and Shibles, 2001). High protein and sulfur-containing amino acids in soybean meal contribute to its nutritional value (Wilcox and Shibles, 2001; van Kempen et al., 2002) while oligosaccharides, including raffinose and stachyose, have detrimental effects on the nutritive value (Wilcox and Shibles, 1994) such that the quantity of soybean meal fed must be limited to avoid flatulence in dogs and digestive disturbances in baby pigs and chicks (Hartwig et al., 1997; van Kempen et al., 2002). In addition, Slominski et al. (1994) and Parsons et al. (2000) report that poultry obtain greater amounts of metabolizable energy from soybean and canola meal with low raffinosaccharide content compared to meal containing moderate levels of raffinosaccharides.

Total sugar content of soybean seeds is also considered to be important for the quality of some soyfoods (Geater and Fehr, 2000). At the same time, raffinose and stachyose have been implicated as flatus-producing factors in soybean seeds (Hymowitz et al., 1972; Jones et al., 1999; Hymowitz and Collins, 1974) which has contributed to their classification as antinutritional compounds.

Accumulation and Degradation of Soluble Sugars

Sucrose is a temporary storage form of glucose, fructose, and carbon that is transported to the seed from green portions of plants during seed development (Dey and Dixon, 1985). The amount of sucrose in seeds decreases rapidly after germination as invertase enzymes digest sucrose to release glucose and fructose which are utilized in the creation of new cells in the growing embryo.

Increased activity of sucrose synthase and alkaline invertase, enzymes involved in sucrose metabolism and catabolism, correspond to accumulation of 90% total dry matter in soybean seeds (Lowell and Kuo, 1989). A change in sugar partitioning during late seed development is accompanied by two to threefold increases in galactinol content as well as a substantial decrease in *myo*-inositol which acts as a substrate for galactinol synthase (Lowell and Kuo, 1989). Highest levels of galactinol synthase activity,

observed at the end of linear seed fill, preceded the accumulation of the highest stachyose levels (Lowell and Kuo, 1989).

RFOs accumulate in nearly all plant seeds during development and disappear during germination (Peterbauer and Richter, 2001). Yazdi-Samadi et al. (1977) observed a steady increase of water-soluble oligosaccharides during soybean seed development. Raffinose and stachyose were not detected until 40-50 days after flowering.

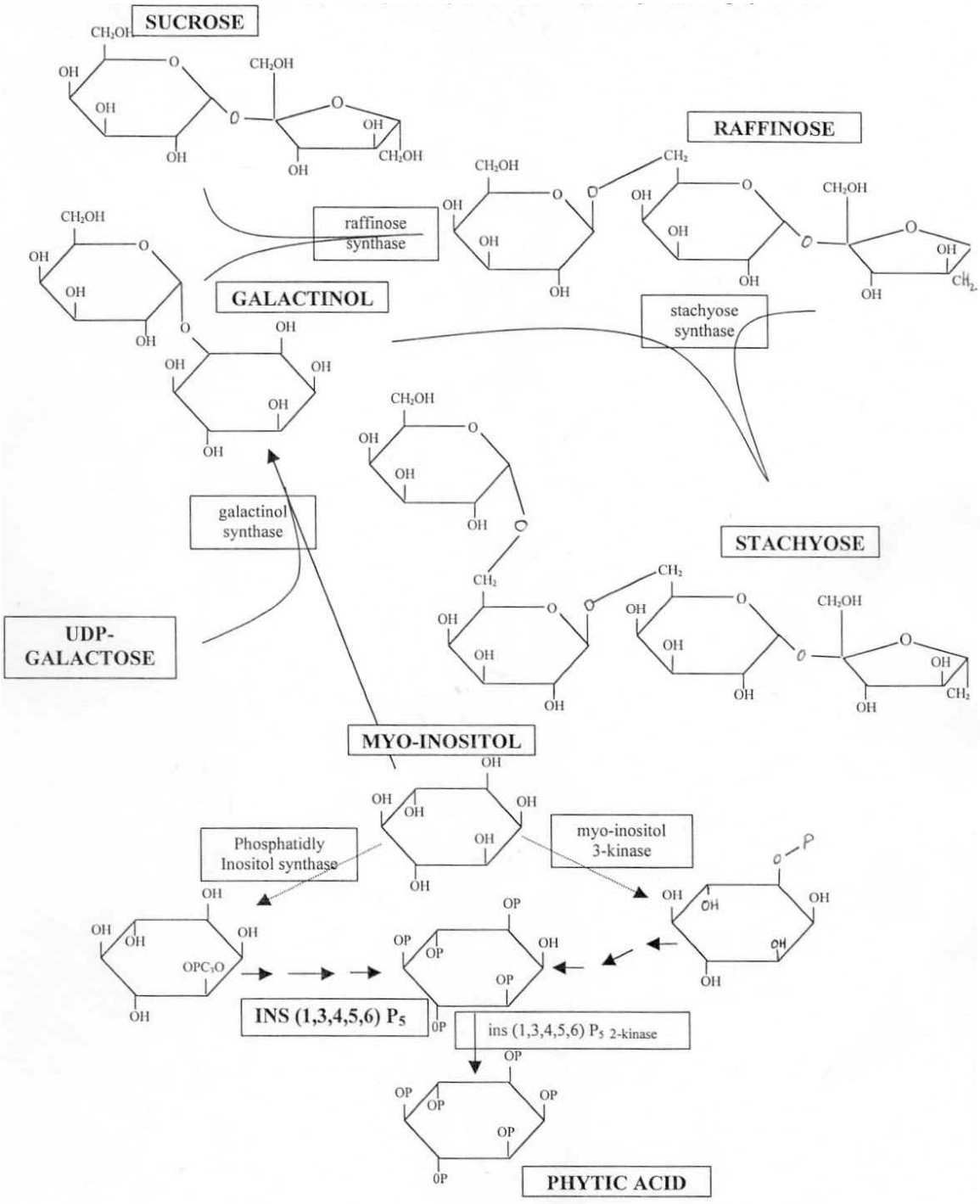
Openshaw et al. (1977) conducted a study to determine the distribution of soluble oligosaccharides in the soybean seed. They found that sucrose was highest in the portion of the seed containing the root:shoot axis and lowest farthest from the root:shoot axis. Stachyose was lowest in the central part of the seed and there were approximately equal amounts present at the root:shoot axis and on the opposite side of the seed. Raffinose appeared to be distributed equally throughout the embryo.

Wolf et al. (1982) reported that temperature has a greater effect on sucrose content than on the amount of raffinosaccharides accumulated. Sucrose concentration was observed to decrease by 56% when the temperature was increased from 18 to 33°C during the day and 13 to 28°C at night. Stachyose showed a slight reduction with increased temperature while raffinose remained unchanged.

The major pathway for biosynthesis of raffinose is via a trans-D-galactosylase-catalysed reaction that requires sucrose and galactinol. Raffinose biosynthesis proceeds by addition of a galactosyl unit to sucrose; this galactosyl unit comes from galactinol, a derivative of myo-inositol (Peterbauer and Richter, 2001). The next member of the RFO is stachyose which requires the addition of a galactose residue to raffinose. Galactinol synthase initiates the committing step of the RFO pathway. Figure 1 shows the biosynthetic pathway proposed by Peterbauer and Richter (2001) for the formation of raffinosacharides.

Lowell and Kuo (1989) report a linear relationship between galactinol formation and raffinosaccharide content. More complex RFOs are synthesized by galactinol-

Figure 1: Proposed biosynthetic pathways for raffinose, stachyose, and phytic acid.



dependent galactosyltransferases or by transfer of galactosyl units between two RFO molecules; galactosyl cyclitols can replace galactinol as galactosyl donors for the formation of stachyose (Peterbauer and Richter, 2001). Therefore, regulation of raffinosaccharide accumulation in developing soybean seeds may depend on galactosyl transferase activity (Lowell and Kuo, 1989).

Soluble sugar content has been observed to decrease as seeds age. However, Yaklich (1985) observed only minor changes in sucrose, raffinose, and stachyose contents of soybean seeds due to field weathering and concluded that field weathering of seeds does not promote changes in the quantity or utilization of soluble oligosaccharides. Total sugar and starch content decreases with many food preparation treatments, such as soaking in water and/or sodium bicarbonate solution, cooking soaked seeds, autoclaving soaked seeds, germination, and frying germinated seeds (Jood et al., 1986). Jood et al. (1986) suggest that germination of pulses for 24 hours is a reasonably good treatment for reduction of flatus-producing carbohydrates without losing the available carbohydrates. Total oligosaccharide content decreased from 3.3% in black beans and 7.5% in soybeans to 0.65% and 1.6%, respectively, through traditional preparation of wari which consists of crushing and fermenting the beans before forming the mixture into wari cakes (Tweary and Muller, 1991).

Function of Raffinosaccharides

The RFO serve as transport metabolites in many legumes (Jones et al., 1999) and have been proposed to play a positive role in cold acclimation and in conferring desiccation tolerance during seed maturation (Dey and Dixon, 1985; Jones et al., 1999; Bentsink et al., 2000). Jones et al. (1999) suggest that these characteristics may be the result of RFO protecting membrane-bound proteins; Bentsink et al. (2000) suggest that the viscosity of oligosaccarides may be responsible for the protection of membranes. Peterbauer and Richter (2001) report that the protective role of raffinosaccharides is only present in *in vitro* systems and thus RFO are probably not completely responsible for the desiccation tolerance of crop seeds. Raffinosaccharides present in cotyledons of dormant seeds appear to be hydrolyzed during germination (Kuo et al., 1977; Peterbauer and

Richter, 2001). However, seed storability was not affected by RFO or sucrose content in *Arabidopsis* seeds (Bentsink et al., 2000).

Genetics of Soluble Sugar Content

One major QTL was found to govern seed stachyose content in *Arabidopsis* accessions with low RFO content (Bentsink et al., 2000). In addition to the nearly monogenic segregation patterns of stachyose content, they also identified three smaller QTLs that control saccharide content in *Arabidopsis*. These three QTL were each related to an individual sugar. The one for stachyose had the largest effect while the one for sucrose had the smallest effect when evaluated in an additive manner. Maughan et al. (2000) reported QTLs controlling 6.1 to 12.4% of the total phenotypic variation for sucrose content in soybean. Of the seven genomic regions identified as having significant effects on sucrose content, three were significantly associated with variation of protein content and three were associated with significant variation of oil content (Maughan et al., 2000).

The major locus controlling stachyose content in *Arabidopsis* seeds with low levels of the RFO also appears to play a role in decreasing raffinose and increasing sucrose content (Bentsink et al., 2000). Comparison of results from various QTL mapping studies for sucrose content with those previously reported for protein and oil content suggests that seed quality traits are either inherited as gene clusters or the major QTLs may control sucrose, protein, and oil content by means of pleiotrophy (Maughan et al., 2000). No significant evidence of epistasis among QTLs controlling sucrose content was observed (Maughan et al., 2000).

Openshaw and Hadley (1978) did not observe cytoplasmic effects in the raffinosaccharide content of soybean seeds from reciprocal F_1 plants derived from crosses of high stachyose parents but they indicated that selection for sugar content among single seeds would be inferior to selection among individual plants or plant progenies because they found little evidence that a seed's genotype influences its sugar content. In contrast, Bentsink et al. (2000) reported maternal effects playing a role in raffinose and stachyose content of *Arabidopsis* seeds derived from reciprocal crosses between high and low stachyose parents. In 1981, Openshaw and Hadley reported that the maternal parent contributes the genetic control of sugar concentration in soybean seeds. There was no

indication of genotype x year or genotype x environment interaction for total sugar content of either population studied; the parents of both populations had intermediate amounts of total sugar (Openshaw and Hadley, 1981).

Openshaw and Hadley (1981) calculated a heritability estimate for total sugar content of 0.38 by regressing the F_3 progeny on their F_2 parents for a population derived from a cross between a high protein parent with a moderate amount of total sugar and another line having both intermediate protein and sugar content. The realized heritability and the heritability estimate calculated from variance components were similar to the estimate calculated by parent offspring regression. An observed range of total sugar (6.2-16.6%), sucrose (3.0-10.2%), raffinose (0.4-1.8%), and stachyose (1.2-3.8%) content indicates the possibility of modifying sugar components in soybeans (Hymowitz and Collins, 1974).

Quantification of Raffinosaccharides

Various methods of determining the amount of carbohydrates in plant material have been used. Some of these include: gas-liquid chromatography of trimethylsilyl derivatives (Openshaw et al., 1977), high performance liquid chromatography (HPLC) (Kuo et al., 1988; Black and Bagley, 1978; Kennedy et al., 1985), near infrared reflectance spectroscopy (NIRS) (Giangiacomo et al., 1981), paper partition chromatography and colorimetry (Tweary and Muller, 1991), high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Jones et al., 1999), cation-exchange chromatography (Gulewicz et al., 2000), and acid hydrolysis (Geater and Fehr, 2000).

Several extraction methods have been employed for use with the various types of analysis. Sugars have been extracted from soy flour using an ethanol-water solution and separation of the oligosaccharides accomplished by injecting the samples onto an HPLC equipped with a carbohydrate column (Black and Bagley, 1978). A procedure employing aqueous extraction of meal, and immediate analysis of extracts without further treatment other than ultrafiltration was found to be much more convenient than gas-liquid chromatography of derivatized sugars (Kennedy et al., 1985). Gulewicz et al. (2000) used a method that includes imbibition of seeds, extraction with 50% ethanol, precipitation of

RFOs, purification of RFOs on diatomaceous earth and charcoal, and cation-exchange chromatography. This method yields high purity RFO preparations in the form of fine, white powder (Gulewicz et al., 2000).

In 1980, Black and Glover noted that the methods of total sugar analysis reported in the literature were labor intensive and time consuming; this was echoed by Geater et al. (2001). Black and Glover (1980) reported that alcohol extracts need further treatment to avoid prematurely reducing chromatography column life and described an extraction method that involves dissolving soluble oligosaccharides in water rather than alcohol. Geater et al. (2001) modified a previously reported method by reducing the amounts of reagents used, eliminating sample filtering, reducing the number of sample dilutions, and increasing the rate of acid hydrolysis (2001). Significant differences between the original and modified methods were observed but a significant sample X method interaction was not observed. Phenotypic correlation of the samples between the original method and the modified extraction method was 0.91 (Geater et al., 2001). The number of samples that could be analyzed in one day increased from 12 to 70 and the cost per sample was reduced from $5.34 to $0.92 with the modifications implemented by Geater et al. (2001).

Black and Glover (1980) reported the importance of acetonitrile concentration for both the extraction and detection processes. The optimum amount of acetonitrile for precipitating proteins was determined to be 1 part acetonitrile:1 part soy extract. They noted that increasing the mobile phase acetonitrile concentration increases column resolution which can compensate for deterioration of the column over time and help to separate closely related sugars like glucose and galactose (Black and Glover, 1980).

Response of refractive index detectors decreases with increased acetonitrile concentration; nonlinear change in detector response between sugars indicates the need for standardization of individual sugars, particularly when the mobile phase exceeds 70% acetonitrile (Black and Glover, 1980).

Extraction in 80% ethanol and methanol was strongly influenced by the extraction temperature and maximum extraction was achieved at the boiling point of each (Johansen et al., 1996). Extraction in water and 50% ethanol or methanol was less heat sensitive than extraction in 80% alcohol and gave comparable results. Ethanol and methanol were equally effective extractants at 50% but ethanol was less effective than methanol at 80%.

In addition, there was no consistent difference observed in the extraction yield when comparing reflux and constant stirring with water bath and occasional mixing.

These extraction procedures typically require grinding or crushing the material from which sugars are to be extracted. A significant interaction between sucrose content in particular parts of the seed as well as among various cultivars indicates that selection for differences in sucrose content based on analyzing only parts of a cotyledon might be unreliable (Openshaw et al., 1977).

Near infrared reflectance spectroscopy has been used to measure concentrations of fructose, glucose, and sucrose for dried apple tissue (Giangiacomo et al., 1981). Correlation coefficients for actual vs predicted values were 0.995 for fructose, 0.994 for glucose, and 0.986 for sucrose (Giangiacomo et al., 1981). This indicates the potential usefulness of NIRS for prediction of sugar content of other plant materials.

Description of Phytic Acid

Phytic acid [*myo*-inositol hexakis phosphate] accumulates in the seeds of all terrestrial plants. Because phytic acid is a reactive compound that readily complexes free cations, phytic acid is rarely observed in plants. Instead, we detect the complexed forms of phytic acid which are collectively called phytates.

Biosynthesis of Phytic Acid/Phytate

Buchanan et al. (2000) and Raboy et al. (2001) describe several possible pathways for the biosynthesis of phytic acid, which rapidly becomes deposited as phytate. Buchanan et al. state that *myo*-Inositol is converted to *myo*-Inositol 1-P in a reversible reaction with *myo*-Insoitol 1-P eventually becoming phytate while Raboy suggests that *myo*-Inositol 1-P is converted to *myo*-Inositol which becomes phytate. Buchanan et al. proposed three possible pathways for *myo*-inositol 1-P to be converted to phytic acid while Raboy proposed two potential pathways for *myo*-inositol to become phytic acid. All of the pathways have been proposed with little evidence to favor any particular one over the others so the actual pathway(s) for phytic acid synthesis remain(s) to be resolved. However, some components are common to several of the proposed pathways. Two of the intermediate compounds of the pathways proposed by Buchanan et al. overlap

with the two compounds Raboy suggests are formed from *myo*-inositol (Phosphatidly inositol and Ins(3)P_1) and the last step on the way toward phytic acid production in Raboy's proposed processes corresponds to the final step in one of the potential pathways presented by Buchanan et al (Ins (1,3,4,5,6)P_5 is converted to phytic acid by Ins (1,3,4,5,6)P_5 2-kinase). Figure 1 shows the two proposed pathways summarized by Raboy et al. (2001).

Myo-inositol is a precursor of both phytic acid and the raffinosaccharides. In the case of phytic acid, *myo*-inositol appears to be a required precursor while there are alternative sources of the galactosyl residues needed for the formation of raffinosaccharides.

Function of Phytic Acid/Phytate

Phytate phosphorus, usually found as a mixed salt of phytic acid, is the principal storage form of phosphorus in most plant seeds (Denbow et al, 1995). Phytate is also thought to be important for various cellular functions such as repairing double-strand breaks in DNA, exporting RNA from the nucleus, and generation of ATP (Raboy, 2001).

Raboy (2001) reports that natural levels of phytic acid are not essential to seed function because maize and barley seeds with low levels of phytic acid were just as viable as seeds containing normal levels. Raboy suggests this may be attributable to the domestication of crop plants such that they do not need to store a lot of phosphorus to perform optimally. However, he also reported that mutants containing less than 5% of the wild type levels of phytate phosphorus experienced more than 15% yield reductions.

Chickens and other monogastric animals do not have endogenous phytase enzymes and thus, are not able to utilize phytate phosphorus efficiently. In addition to making phosphorus unavailable for use by monogastric animals, many elements such as zinc, copper, cobalt, manganese, iron, and calcium can be bound by phytic acid with zinc and copper having the highest binding affinity (Radcliffe, 1997; Hortin et al, 1993). Starch and protein are also known to be complexed by phytate. This binding of mineral compounds is generally recognized to cause nutrient deficiencies, but Harland and Morris (1995) suggest the binding of minerals by phytate may have anticarcinogenic effects as well. Studies regarding potential benefits of phytate are preliminary and further work is

being done but, regardless of the health benefits that may be discovered, the binding of essential minerals leading to nutrient deficiencies in monogastric animals has been well documented so the benefits of phytate may only be realizable in ruminant animals such as cattle.

Phosphorus absorption appears to increase when humans and monogastric animals consume low phytate products rather than conventional meal and bran products. Adding phytase to corn-soy rations to reduce the levels of phytate resulted in less zinc deficiency in swine (Lei et al., 1993), and human consumption of low phytate wheat bran appears to result in a higher level of phosphorus absorption than human consumption of conventional wheat bran (Morris et al., 1988).

Genetics of Phytic Acid/Phytate Content

Seeds of most species exhibit a strong positive relationship between total seed phosphorus and phytate phosphorus (Raboy, 2001). Non-GMO mutants in which this relationship is altered have been identified in maize, barley, and rice. These mutants have altered proportions of phosphorus forms rather than alterations in the total amount of phosphorus present (Raboy et al., 2001).

Recessive alleles at two loci in maize, *lpa1*-1 and *lpa2*-1, were discovered in the first mutants found to have less than normal amounts of phytate phosphorus in comparison with the total phosphorus content (Raboy, 2001). Many additional *lpa1* alleles have been identified in maize (Raboy et al., 2000), barley (Rasmussen and Hatzack, 1998), rice (Larson and Raboy, 1999), and soybean (Wilcox, 2000).

The *lpa1*-1 mutation reduces phytate phosphorus content by two thirds and results in an equimolar increase of inorganic phosphorus. Meanwhile, the *lpa2*-1 mutation decreases phytate phosphorus by about 50% and increases levels of both intermediate inositol phosphates and inorganic phosphorus (Raboy, 2001). These patterns indicate that the two mutations are interrupting different steps in the biosynthetic pathway with *lpa1*-1 interfering at an early stage and *lpa2*-1 functioning at a later stage (Raboy, 2001). Such mutants have been used in studies to define the biochemical pathways related to phytic acid.

Quantification of Phytate Phosphorus

Most of the phosphorus stored in seeds is in the form of phytate which is not free phosphorus so extraction procedures must release phytate phosphorus such that it can be measured. In addition to digesting phytate phosphorus into available phosphorus, many extraction methods also yield various levels of intermediate inositol phosphates which must be separated to determine the amount of phytate phosphorus present. Some methods used to separate inositol phosphates are colorimetry, low pressure ion exchange column chromatography, phosphorus-32 Fourier transform nuclear magnetic resonance (NMR) spectroscopy, near-infrared reflectance spectroscopy (NIRS) and high pressure liquid chromatography (HPLC) (Harland and Oberleas, 1977).

Harland and Oberleas (1977) proposed a method for determining the amount of phytate phosphorus in textured vegetable proteins which involved concentrating the phyate on an anion exchange resin, removing any possible contaminating inorganic phosphate and eluting the phytate. The resulting phytate was quantified by use of an ion-exchange procedure. More recently, development of HPLC instrumentation has enabled separation and quantification of inositol phosphates via HPLC analysis which is much more rapid and convenient than using ion-exchange gradients for quantification of phytate (Harland and Morris, 1995).

Interrelationships of Seed Components

Openshaw and Hadley (1981) reported that low total sugar content was associated with decreased yield in some soybean generations from crosses between high protein and conventional lines. However, Wilcox and Shibles did not observe an association of carbohydrate concentrations with seed yield in various lines with protein content ranging from 413 g/kg to 468 g/kg on a dry weight basis (2001).

Simple correlation analysis revealed that total sugar content and oil content in soybean seeds are positively associated, and each was negatively correlated with protein content (Hymowitz et al., 1972). Wilcox and Shibles (2001) also observed that protein increased at the expense of oil, total carbohydrates, and sucrose. Sucrose and raffinose content were positively correlated with oil content while stachyose content was positively associated with protein (Hymowitz et al., 1972). The correlation between protein and

stachyose+raffinose was negative, but nonsignificant (Hartwig et al., 1997). Sugar was positively associated with oil and negatively associated with protein and the sum of oil and protein (Openshaw and Hadley, 1981). Seed size had a major influence on the association of total sugar with protein, oil, and fiber individually (Geater and Fehr, 2000).

Total sugar content was more highly correlated with the sum of protein and oil than any of the individual sugars (Geater and Fehr, 2000). Analysis of protein+oil by NIR was not influenced by seed size and appears to be a reliable indicator of total sugar content (Geater and Fehr, 2000). Despite the significant correlation between sugars and oil or protein, some variation in total sugar content remains to be explained (Hymowitz et al., 1972).

Phytate shares several characteristics with the raffinosaccharides; both serve as storage forms of their respective component, neither can be digested by monogastric animals, and both accumulate as seeds mature. These similarities, in conjunction with a proposed common biosynthetic link of *myo*-inositol (Figure 1), have led researchers to speculate that the amount of phytic acid formed may be related to the level of raffinosaccharide content. Additional information adding to the relevance of this speculation came in 2001 when Peterbauer and Richter reported that metabolism of methylated inositols is a potential source of galactysyl residues for the formation of raffinosaccharides.

MATERIALS AND METHODS

Genetic Materials

Selection/Characterization of Parents

Seed of V99-5089 soybean grown in 2000 at Blacksburg, VA was observed to have high sucrose (14.6%) and low raffinose (0.5%) and stachyose (0.4%) contents. V99-5089 was derived from a cross of PI87013 and MFL-552. PI87013 was selected as a parent in the breeding program because of the relatively high sucrose levels reported by Hymowitz et al. (1972) and MFL-552 is also known to have relatively high sucrose content. V99-6180 is a breeding line derived from a cross between Hutcheson and V94-2924 and has slightly lower than average sucrose content. Essex is a cultivar with normal sugar levels.

PI87013, MFL-552, V99-5089, and Essex have purple flowers while V99-6180 contains both white and purple flowers. Brown pods were observed for PI87013, MFL-552, and V99-5089 but Essex and V99-6180 have tan pods. MFL-552 and V99-5089 have yellow hila while the other parental lines have imperfect black hila. All parents have gray pubescence color. See Table 1 for agronomic and quality characteristics of parents used in this study.

Populations Studied

Three segregating populations were evaluated in this study. PI87013 and MFL-552 were crossed to observe the frequency of progeny with the phenotype of V99-5089. V99-5089 was crossed with Essex and V99-6180 to observe the segregation patterns of sucrose and stachyose. These populations were developed from crosses made at Blacksburg, VA during the summer of 2001. Population 1 is derived from a cross of PI87013 with MFL-552. Population 1 consisted of 50 F_2 plants which were derived from six F_1 plants. Population 2, consisting of 100 F_2 plants derived from two F_1 plants, originated from a cross between V99-5089 and Essex. Population 3 resulted from a cross of V99-5089 with V99-6180. Fifty F_2 seeds, derived from four F_1 plants, were planted for population 3.

Table 1: Summary of agronomic and quality characteristics of parents

Parent	MFL-552†	V99-5089†	Essex†	Essex‡	V99-6180‡
Yield (kg/hectare)	3542.7	2494.7	3722.8	3478.4	3156.9
Maturity§	58	54	58	41	40
Height (cm)	71.1	63.5	86.4	88.9	76.2
Lodging score¶	1.8	1.0	1.8	3.2	4.5
Protein (% DW)	43	42.1	NA	43.7	44.2
Oil (% DW)	17.0	17.6	NA	18.9	19.2
Sucrose (% DW)	9.8	14.6	NA	NA	5.6
Raffinose (% DW)	1.4	0.5	NA	NA	1.4
Stachyose (% DW)	3.8	0.4	NA	NA	5.6

† data from preliminary tests grown at Blacksburg in 2000

‡data from preliminary test grown at Warsaw in 2000
§ maturity is presented as the number of days after August 30 that 95% of the pods were mature
¶ 1=completely erect; 5=completely lodged

Plant Growth and Harvest

Up to ten F_1 seeds per cross were planted in the greenhouse on the Virginia Tech campus on October 27, 2001. The soil used in the greenhouse consisted of a 1:1 mixture of field soil and Metro-Mix 360. The Metro-Mix 360 consists of 35-45% medium grade horticultural vermiculite, 31-50% Canadian sphagnum peat moss, 12-25% processed bark ash. The field soil and Metro-Mix 360 were mixed in an electric mixer until homogeneous.

F_1 plants were grown at day/night temperatures of 28/23°C and subjected to 16 h of daylight for the first month after planting and twelve hour days for the remaining time until harvest. Seeds were harvested as they matured and F_2 seeds were planted on February 13, 2002. F_3 seeds were harvested between May 29 and July 3 as they matured. All plants in each generation (F_1-F_3) were allowed to self pollinate naturally.

F_3 seeds were planted in a randomized block design in the field at Blacksburg and Warsaw, VA with six seeds per plot and two replications at both locations; each plot was 0.3 m in length with 1 m between plots and 5 cm between seeds in each plot. Lines from each cross were planted as a block within the first replication, with the parents of each population randomized among the rows of the corresponding population; all three populations and their parents were completely randomized in the second replication. Entries that did not have enough seed for four plots were substituted with soybean cultivar Camp. Essex was included in each of the three populations as a check cultivar.

The plots at both locations were fertilized with 67 kg P_20_5 per hectare. In addition, K_2O fertilizer was applied at a rate of 90 kg/hectare in Blacksburg and 67 kg/hectare in Warsaw. These plots were planted by hand on July 5 at Blacksburg and July 8 at Warsaw. There was a lack of adequate rainfall during the summer of 2002 so both locations were irrigated.

Observations recorded during the growing season for all plots at both locations included: flower, pubescence, and pod color, mature plant height, maturity date, and lodging score. Flower color was observed at bloom while pubescence and pod color were observed after maturation. Mature plant height was measured and recorded as an average height, in centimeters, for each plot. Maturity date was recorded as the number

of days after August 31 when 95% of the pods were brown. Lodging scores were assigned on a scale of 1 to 5 with 1 being non-lodged plants and 5 being completely lodged plants. In addition, the number of plants in each plot was recorded early in the season for all plots and verified later in the season for plots of population 2.

Twenty nine F_3 families from population 2 (V99-5089 x Essex) were harvested as individual plants at Blacksburg to asses the variation among plants of a given family; only families that had at least six vigorous plants among the two replications were chosen. Because it was not feasible to harvest all families as individual plants, the remaining plots at both locations were harvested in bulk.

Sampling Procedure

Remnant F_3 seeds from the field planting of population 2 were evaluated for sugar content; immature seeds were not included. Five-gram samples of seed from each individually harvested F_3 plant and 50-seed samples from the bulk harvested $F_{2:3}$ were ground for sugar analysis. Sugar analyses were conducted on plots of all 3 populations harvested at Blacksburg. Limited availability of the HPLC machine forced a reductin in the analysis of the Warsaw samples. All plots from population 2 and the parents of all crosses were analyzed as well as a few samples from populations 1 and 3 which had missing or questionable data from the Blacksburg analysis.

Optimizing Soluble Oligosaccharide Extraction

The amount of seed available for sugar analysis from this study was less than the amount of seed ground to evaluate sugar content of breeding lines, therefore, an adequate procedure for grinding smaller samples needed to be determined. The grinders available for use in this study were the C&H Laboratory Grinder Size 8 and the Cyclone Sample Mill with various sized screens for each. The Cyclone Sample Mill is more convenient for use with smaller samples but also produces smaller particles with a larger surface area than any of the screens on the Laboratory Grinder Size 8. For this reason, sugar was extracted from particles passing through three screens on the Laboratory Grinder Size 8 (3.0, 2.5, and 1.0 mm) and a 1.0 mm screen on the Cyclone Sample Mill to determine the effect of particle size on the amount of sugar extracted. Because the 1.0 mm screen on

the C&H Laboratory Grinder Size 8 (size 3) is used for grinding seed of breeding lines for sugar analysis, this was used at the standard to which the other sizes were compared.

It was hypothesized that sugar could be extracted more efficiently from smaller particles so one-gram samples of each particle size were extracted for 15, 30, or 60 minutes and the extracts were analyzed by HPLC. The experimental design of this study was a 3x4 factorial treatment with two replications. Each replication was a separate extract from the same sample of ground soybean seed.

Soluble sugars were extracted from 1.0, 0.75, 0.5, and 0.25 g of ground seed to determine the effect of sample size on sugar analysis. Seed samples (75 g) from a single lot each of Hutcheson and V99-5089 were ground using the 1.0 mm screen on the C&H Laboratory Grinder Size 8. Sugar was extracted from two samples of each size and they were run on the HPLC in non-randomized complete blocks. Analyses of variance and contrasts were conducted to determine if there were significant differences in the percent sugar calculated on a dry weight basis for different sized samples from the same lot of ground seed.

Laboratory Procedures

Soluble Oligosaccharide Extraction and Analysis

Soluble sugars were extracted using a method developed by P. Chen and M. Cicek at Virginia Polytechnic Institute and State University (Cicek, 2001) because the supplies and instrumentation required for analysis by this method were available. The procedure involves extracting soybean meal in double distilled water and removing all components other than soluble oligosaccharides via acetonitrile precipitation, evaporation and centrifugation.

Approximately 1 g of ground sample was weighed and placed in a glass tube with 10 mL double distilled water and “vortexed”; tubes were then placed on a horizontal shaker for 20 min. Samples were centrifuged for 8 min at 200 rpm to separate the non-soluble components. After centrifugation, 7 mL acetonitrile and 5 mL of the supernatant were combined in a clean glass tube. This solution was left stationary at room temperature for 2 h to precipitate water-soluble proteins. A portion of the liquid was

decanted into a 1.5 mL eppendorf tube and micro-centrifuged for 15 min at 14,000 rpm. The supernatant (mL) was pipetted into a reactotherm vial, dried with air at 80ºC, and resuspended in 400 µL of 65% acetonitrile. This suspension was transferred to an eppendorf tube, centrifuged, and the soluble portion was transferred to a Fisher Scientific brand target DP vial for HPLC analysis. For convenience, a maximum of 22 samples was extracted at a time (the centrifuge held 24 tubes; 22 samples and two control samples). The control samples used for all analyses were taken from a single lot of MFL-552 and used to normalize data when a particular run was more than two standard deviation units away from the average of all other observations. This was done by averaging all the observations of MFL-552 and bringing the significantly different values to that average by using the necessary conversion factor which was then applied to all data from that run.

Moisture content was determined by the difference in weight of samples weighed at the time of extraction and after oven drying. Because all samples were allowed to equilibrate to the same temperature and moisture, samples (2 g) from four random ground samples were evaluated to obtain average moisture content for each run of samples.

An HPLC system utilizing an HP1047A refractive index detector operated by the March 2000 edition of Agilent Technologies ChemStation *Plus* equipped with an astec 250x4.6 mm NH_2 polymer column preceded by a 5 µ 10x4.6 mm guard column was used for quantification of soluble saccharides in the extracted samples. Three concentration standards were prepared from stock solutions to create a standard curve to be used for integration of the sample curves. Stock solutions were 5% solutions of sucrose, raffinose, and stachyose prepared by dissolving purified sugar powder, obtained from Sigma-Aldrich, in double distilled water. Standard 1 contained 2.5 mg/mL sucrose, 1.25 mg/mL raffinose, and 1.25 mg/mL stachyose. Standard 2 was twice as concentrated as standard 1 and standard 3 was twice as concentrated as standard 2. Ten milliliters of stock solution were prepared at a time and stored in the refrigerator; new standards were prepared with each run of samples. Integration values (mg/mL) obtained were converted to percent dry weight (DW) by the following formula which takes into account the dilutions that occur throughout the extraction process as well as the initial sample weight and moisture:

(1) (2) (3) (4) (5)

[[ChemStation value (mg/ml) x .01 x 40 x 12 x 2] ÷ 10] ÷ dry wt. (g) = % CHO

(1) multiply by .01 to convert mg/mL to mg of sugar injected on HPLC; 10 µl x .01 = mL

(2) multiply by 40 because 10 µL of a 400 µL solution is injected on the HPLC

(3) multiply by 12 because 1 mL of the 12 mL solution is used for the remaining steps of the extraction procedure

(4) multiply by 2 because 5 mL of the initial 10ml solution is used for extraction

(5) divide by 10 to convert mg sugar/g sample to % sugar on a dry wt basis

Phosphorus Extraction and Tests

Sixty remnant samples from families of crosses 1 (PI87013 x MFL-552) and 2 (V99-5089 x Essex) having high, low, and intermediate stachyose content were chosen to be analyzed for inorganic and phytate phosphorus. F_2 plants grown in the field at Blacksburg in 2002 were used for cross 1 because there were only 2 low stachyose $F_{2:3}$ families from population 1. The samples from cross 2 were the same samples analyzed for sugar content which consisted of seed from $F_{2:3}$ plants grown in the field at Blacksburg in 2002. This was a preliminary study and, therefore, the analyses were not replicated. Total phosphorus was first extracted from the ground sample by adding 10 mL of a 0.4 N HCl:0.7 M Na_2SO_4 solution to 0.2 g of sample and shaking overnight. Samples were then centrifuged to separate the soluble and insoluble fractions. From this supernatant, two independent samples were taken, one for inorganic phosphorus and the other for phytate phosphorus analysis.

<u>Inorganic Phosphorus (Pi) Assay</u>

Supernatant (1 mL) was filtered into an eppendorf tube and stored in the refrigerator until analyzed. The analysis, a modified version of the Chen et al. (1956) procedure uses only 10 µL of this solution. Modifications to Chen et al (1956) described by Dan Israel,USDA-ARS, Raleigh, NC, (personal communication) involve scaling down the volume such that the reactions can be performed in microtiter plates and not heating

the colorimetric assay. Additional modifications include eliminating the 12.5% TCA:25 mM $MgCl_2$ and reducing the developing time to 30 minutes. Samples were pipetted into a microcuvette and quantified by a spectrophotometer at 820 nm. Absorbance readings for standard solutions with 0, 0.31, 0.62, 0.93, 1.24, and 1.55 µg phosphorus were used to calculate a conversion factor that was used to extrapolate the amount of phosphorus (µg) in the soybean extracts. The following formula was used to convert absorbance values to % Pi:

(1) (2)

spectrophotometer reading (nm) x conversion factor (µg/nm) x 100 x 10 ÷ 1,000,000 (µg/g) ÷ dry weight (g) = g Pi/g seed dry weight

(1) multiply by 100 to account for using 10µl from the 1ml sample
(2) multiply by 10 to account for taking a 1ml sample from the initial 10ml solution

Phytate Phosphorus(PP) Assay

The extraction method was adapted from that described by Raboy et al. (1990) and the modified version of Chen et al.'s (1956) method mentioned previously was used for visualization and quantification. The modifications to Raboy et al (1990) involved decreasing the amount of reagents to fit the reaction vessels available.

Supernatant (3 mL) was filtered into a borosilicate tube to which 3 mL 15 mM $FeCl_3$:0.2N HCl were added before heating in a dry bath block at 100 C for 30 min to coagulate and precipitate proteins. Samples were transferred to Corex tubes, centrifuged for 10 min at 12,000 rpm and the pellet was washed with 5 mL 0.2N HCl twice before it was dissolved in 2 mL H_2SO_4 and wet ashed with 2 mL H_2O_2. The remaining material was resuspended in 1 mL de-ionized water, transferred to an eppendorf tube, and refrigerated until analysis by modified Chen's procedure as described above. The following formula was used to convert absorbance values to % PP:

(1) (2)

spectrophotometer reading (nm) x conversion factor (µg/nm) x 100 x 3.333 ÷ 1,000,000 (µg/g) ÷ dry weight (g) = g PP/g seed dry weight

(1) multiply by 100 to account for using 10 µl of a 1 mL
(2) multiply by 3.333 to account for taking a 3 ml sample for the initial 10 ml solution

Statistical Analysis

Qualitative Genetics Study

F_2-derived families and F_3 plants were classified as having either high or low stachyose content, based on observation of all data, and tested for goodness of fit to Mendelian ratios to test the hypothesis that stachyose content is simply inherited.

Quantitative Genetics Study

Variance components of sucrose, raffinose, and stachyose content for the homozygous high and low families of individual plants harvested from population 2 at Blacksburg and means of probable homozygous high and low bulk harvested plots of population 2 from both Blacksburg and Warsaw were estimated. Because plants were not harvested individually from populations 1 and 3, it was not possible to differentiate between the heterozygous and homozygous high plots so these populations were not evaluated quantitatively. Variance components were generated by the mixed models procedure of SAS using a model that included fixed effects of block and random effects of entry. Additive variance (V_A) estimates were obtained directly from the SAS results and environmental variance (V_E) was derived from the statistical variance components using the formulae shown in Table 2. Narrow sense heritability estimates were calculated as described by Kearsy and Pooni (1996) and shown in Table 2.

Separate analyses were conducted for homozygous high and low stachyose groups of F_2 families to avoid confounding the heritability estimates with the major gene effects. The low stachyose group included families with less than 1% stachyose on a dry weight basis and the homozygous high stachyose families were identified by comparing family means for the individual plant families with the bulk harvested families. Families in which all individual plants were observed to have either high or low stachyose levels were assumed to be homozygous while those containing plants with both high and low stachyose content were considered heterozygous for stachyose content. Families with

Table 2: Derivation of genetic variance components and heritability estimates from statistical variance components

Source	df	MS	ems	Genetic variance components
Between families	n-1	MSB	$\sigma^2_W + r\sigma^2_B$	$\sigma^2_B = V_A$
Within families	r(n-1)	MSW	σ^2_W	$\sigma^2_W - ½(V_A) = V_E$

Heritability estimates

$h^2 = V_A/(V_A+V_E)$

stachyose content equal to or higher than the lowest average of the families known to be homozygous high stachyose, based on individual plants, were assumed to be homozygous for high stachyose content; these were families with greater than 4% stachyose. Families with less that 1% stachyose were considered to be homozygous low for stachyose content based on overall stachyose distribution observed in this study.

Relationship Among Soybean Seed Components and Agronomic Traits

A correlation of means from the 29 families of population 2 analyzed as individual plants was conducted in AgroBase to determine the relationship among the soluble oligosaccharides. Agronomic traits were analyzed for the putative homozygous high and homozygous low stachyose families; putative homozygous families were identified in the manner discussed previously. All entries with any missing data were omitted and population 1 was not analyzed because there were only two low stachyose families. Agronomic and sugar data from Blacksburg were used for this analysis because sugar data were not available from Warsaw for population 3. Orthogonal contrasts were performed using SAS to determine the significance of average differences between the high and low stachyose families.

RESULTS AND DISCUSSION

Optimization Experiments

The amount of seed available for sugar analysis from this study was less than the amount of seed ground to evaluate sugar content of breeding lines, therefore, an adequate procedure for grinding smaller samples needed to be determined. The grinders available for use in this study were the C&H Laboratory Grinder Size 8 and the Cyclone Sample Mill with various sized screens for each. The Cyclone Sample Mill is more convenient for use with smaller samples but also produces smaller particles with a larger surface area than any of the screens on the Laboratory Grinder Size 8. For this reason, sugar was extracted from particles passing through three screens on the Laboratory Grinder Size 8 (3.0, 2.5, and 1.0 mm) and a 1.0 mm screen on the Cyclone Sample Mill to determine the effect of particle size on the amount of sugar extracted. It was hypothesized that sugar could be extracted from smaller particles so sugar was extracted for 15, 30, and 60 minutes from samples of each particle size.

The 3.0, 2.5, and 1.0 mm screens used on the C&H Laboratory Grinder Size 8 created different sized particles as did the 1 mm screen on the Cyclone Sample Mill. Despite passing through a 1mm screen, based on visual inspection, the particles from the Cyclone Sample Mill appeared to be finer than the particles from the C&H Laboratory Grinder Size 8. Results from this study indicate no differences ($p \geq 0.05$) in estimated sucrose, raffinose, or stachyose as a result of extraction time but significant differences ($p \leq 0.05$) were detected among the four different particle sizes (Table 3). This agrees with the findings of Cicek (2001) who extracted sugar from three soybean lines for 15, 30, 60, and 120 minutes and found no differences in the amount of sucrose, raffinose, or stachyose detected. Analysis indicated that the amount of each sugar extracted from samples of size 1, passing through a 3.0 mm screen on the C&H Laboratory Grinder Size 8, is equivalent to the amount of each sugar extracted from size 2, particles passing through a 2.5 mm screen on the same grinder. Likewise, size 3, particles passing through a 1 mm screen on the C&H Laboratory Grinder Size 8, is not significantly different from size 4, passing through a 1 mm screen on the Cyclone Sample Mill. However, sucrose

Table 3: Mean (n=2) sucrose, raffinose, and stachyose extracted from seed samples of MFL-552.

Extraction time (min)	Sugar	Particle size† 1	2	3	4	Average
15	Sucrose	9.68	9.05	9.81	10.32	9.71
30	Sucrose	8.72	8.86	9.53	9.45	9.14
60	Sucrose	8.58	8.72	10.48	10.84	9.65
Average		8.99	8.87*	9.94	10.20	
15	Raffinose	1.93	1.69	1.99	1.94	1.89
30	Raffinose	1.70	1.72	1.91	1.83	1.79
60	Raffinose	1.70	1.75	2.10	2.19	1.93
Average		1.77	1.72*	2.00	1.99	
15	Stachyose	2.11	1.93	2.24	2.13	2.10
30	Stachyose	1.90	1.91	2.12	2.08	2.00
60	Stachyose	1.79	1.91	2.22	2.39	2.07
Average		1.93*	1.91*	2.19	2.20	

† particle size 1,2 and 3 represent seeds processed using 3.0, 2.5, and 1.0 mm screens on a C&H Laboratory Grinder Size 8 respectively and particle size 4 represents samples passing through a 1 mm screen on a Cyclone Sample Mill

*indicates average values that are different from those of size 3 at a significance of p=0.05

and raffinose extracted from samples of size 2 were significantly lower (Table 3) than from samples of either size 3 or 4 and stachyose was significantly lower from both sizes 1 and 2 than either 3 or 4. The particle size x extraction time interaction was not significant and the two replications were not significantly different from each other. Particle size 3 resulted from using the smallest screen available for the C&H Laboratory Grinder Size 8 which is the grinder and screen commonly used to grind soybean samples of breeding lines for sugar analysis at Virginia Tech. Because size 4, created by the Cyclone Sample Mill, produced similar results, it was determined that the Cyclone Sample Mill could be used to grind the samples for sugar analysis without changing the extraction procedure. Because the Cyclone Sample Mill is better suited to grinding small samples than the C&H Laboratory Grinder Size 8, the Cyclone Sample Mill was used to grind the small samples available for this study.

Because of the small amount of seed available, there was some speculation about using less than 1 g of ground sample for sugar analysis and a study was conducted to determine the effect of sample size on the estimation of sucrose, raffinose, and stachyose content of soybean seeds. Sample size did not have a significant effect on the amount of sugar extracted from either seed lot (Table 4). The amounts of raffinose present in samples of V99-5089 were not detectable. Although these results indicate that samples less than 1 g could be successfully used to evaluate seed lots, it was decided that smaller samples would not allow appropriate representation of the 2 to 6 plants in a plot and only plots yielding at least 1 g of ground sample were analyzed.

Qualitative Genetics Study

Hilum Color

Hilum colors were examined in the context of F_2 progeny from a single F_1 plant as an indication of whether a true cross was made or if a particular F_1 plant resulted from self-pollination. All F_1 plants constituting populations 1 and 2 produced F_2 progeny that segregated for hilum color indicating that these progeny are likely the result of a cross rather than self-pollination. In population 3, all F_2 progeny had yellow hila as expected because both parents have yellow hila (Table 5).

Table 4: Mean (n=2) sucrose, raffinose, and stachyose extracted from 1.0, 0.75, 0.50, and 0.25 g ground soybean seed samples of Hutcheson and V99-5089

	Sample	% dry weight of:		
Cultivar	Size (g)	Sucrose	Raffinose	Stachyose
Hutcheson	0.25	7.84	0.98	4.54
Hutcheson	0.50	8.23	0.90	5.01
Hutcheson	0.75	7.70	0.82	4.59
Hutcheson	1.00	7.95	0.82	4.80
V99-5089	0.25	12.41	0.00	0.36
V99-5089	0.50	13.24	0.00	0.60
V99-5089	0.75	13.17	0.00	0.44
V99-5089	1.00	12.89	0.00	0.40

Table 5: Segregation of stachyose content and hilum color in F_2 families derived from individual F_1 plants

Cross	F_1 plant no.	F_2 hilum color	F_2 families high†	F_2 families low†	Stachyose Mean‡	Stachyose Std.dev.‡	$\chi^2_{3:1}$	p
PI87013 x MFL-552	1-1	seg	6	1	3.12	1.36	0.429	.5-.7
PI87013 x MFL-552	1-2	seg	7	0	4.44	0.39	2.333	.1-.2
PI87013 x MFL-552	1-3	seg	6	1	3.16	1.47	0.429	.5-.7
PI87013 x MFL-552	1-4	seg	7	0	4.04	0.24	2.333	.1-.2
PI87013 x MFL-552	1-5	seg	6	0	4.32	0.45	2.000	.1-.2
PI87013 x MFL-552	1-6	seg	8	0	4.10	0.40	2.667	.1-.2
Population 1			**40**	**2**			**9.175**	**.001-.01**
V99-5089 x Essex	4-1	seg	34	13	2.90	1.87	0.177	.5-.7
V99-5089 x Essex	4-2	seg	33	12	2.62	1.68	0.067	.8-.9
Population 2§			**67**	**25**			**0.232**	**.5-.7**
V99-5089 x V99-6180	8-1	yellow	9	2	3.21	1.52	0.273	.5-.7
V99-5089 x V99-6180	8-2	yellow	9	4	2.78	1.65	0.231	.5-.7
V99-5089 x V99-6180	8-4	yellow	11	2	3.37	1.59	0.641	.3-.5
V99-5089 x V99-6180	8-6	yellow	9	1	3.10	1.03	1.200	.2-.3
Population 3			**38**	**9**			**0.858**	**.3-.5**

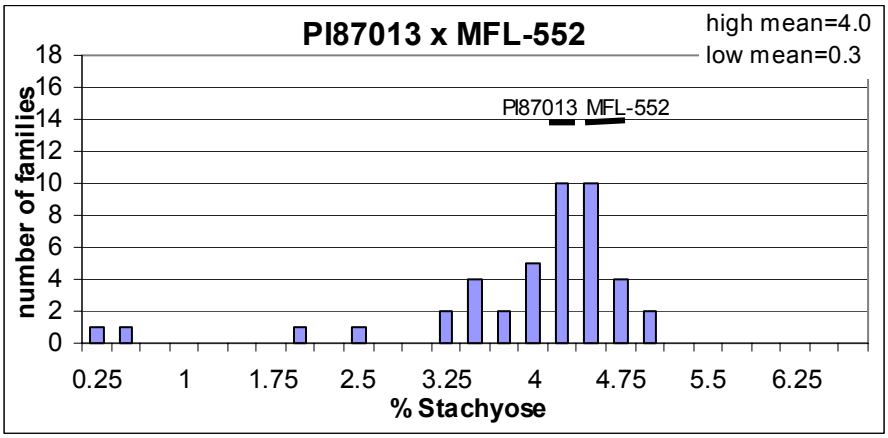
† low<1<high
‡ mean and standard deviation were calculated using only the high stachyose families
§ observed at both Blacksburg and Warsaw

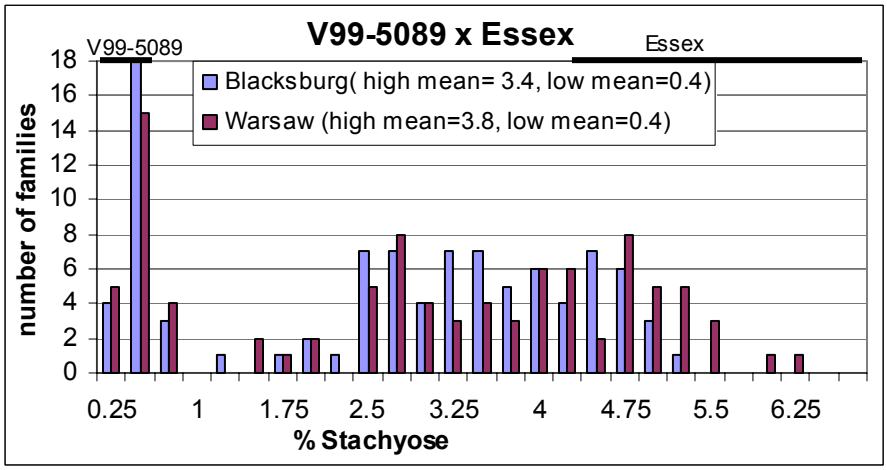
Stachyose Distribution

Stachyose content of $F_{2:3}$ families showed a more distinct bimodal segregation than either sucrose or raffinose content. Therefore, only stachyose data were tested against Mendelian ratios. A range in stachyose content from 0.12% to 6.1% DW was observed for the soybean seeds analyzed from $F_{2:3}$ plants in this study. The stachyose distribution for the $F_{2:3}$ families of each cross was approximately bimodal (Figure 2). There is a small group of families in each cross with low stachyose and a larger group with intermediate to high stachyose levels. There were few families with stachyose levels between 1% and 2%, and for the remainder of this discussion, individuals and family means with stachyose content above 1% will be assumed to have high stachyose while those with less than 1% stachyose will be referred to as having low stachyose.

The parents of the three populations used for this study had stachyose contents ranging from 0.23 to 4.96%; V99-5089 has low stachyose but all other parental lines had high stachyose levels (Table 6). It is interesting to note that the levels of sucrose and raffinose detected in the seeds of Essex were nearly identical in both locations but stachyose was much higher at Warsaw. V99-5089 had relatively similar results from each location but there was considerably less sucrose, more raffinose, and less stachyose in the seed of PI87013 from Warsaw compared to those from Blacksburg. Tables 7, 8 and 9 show that there is not a consistent environmental effect on the accumulation of sucrose, raffinose, or stachyose based on the lack of agreement in rank. However, data from field tests at Blacksburg and Warsaw were generally more similar than the greenhouse data was to either two field location. Sucrose values were lower from the F_2 seeds grown in the greenhouse (range=4.63-8.94%) than from the $F_{2:3}$ seeds from either Blacksburg (range=6.12-12.08%) or Warsaw (range=6.12-11.74%). Meanwhile, the amount of raffinose and stachyose in the F_2 seeds from the greenhouse was generally higher than the amount of raffinose and stachyose detected in $F_{2:3}$ seeds from either field location. This may support the idea that raffinosaccharide accumulation is induced by

Figure 2: Distribution of stachyose content in $F_{2:3}$ families with parental range and means of high (<1%) and low (>1%) stachyose families





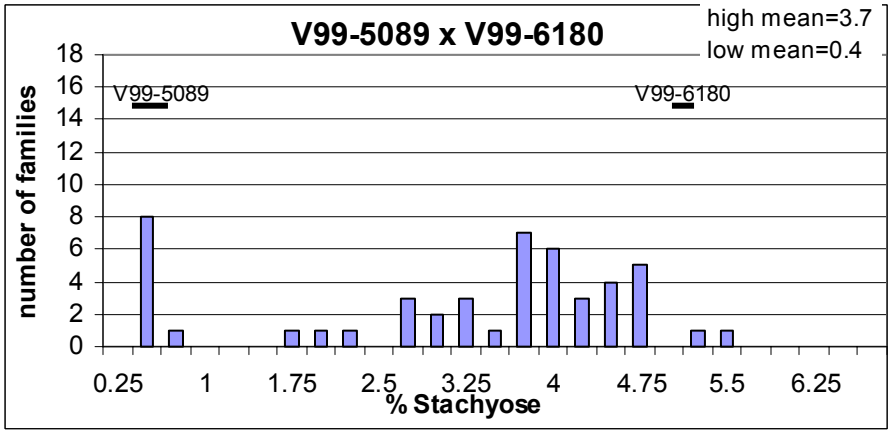


Table 6: Average sugar content of parents grown in 2002 at Blacksburg and Warsaw

Parent	Location†	Population	% Sucrose	% Raffinose	% Stachyose
PI87013	BB	1	8.89	0.81	4.39
PI87013	W	1	5.82	1.03	3.72
MFL-552	BB	1	6.84	0.86	4.15
V99-5089	BB	2,3	11.57	0.36	0.27
V99-5089	W	2,3	10.79	0.29	0.22
Essex	BB	2	6.12	0.64	4.44
Essex	W	2	6.12	0.68	5.48
V99-6180	BB	3	5.74	1.01	5.01

† BB=Blacksburg; W=Warsaw

Table 7: Comparison of sucrose values from F_2 families with $F_{2:3}$ data from Blacksburg and Warsaw and F_2 greenhouse data

	Sucrose (% DW) from:		
F_2 plant	Blacksburg	Warsaw	Greenhouse
Essex	6.12	6.12	--
20-14	6.71	7.23	6.15
20-84	6.71	6.56	6.24
20-48	6.89	6.96	6.22
20-53	6.97	6.90	5.77
20-35	7.16	7.14	4.93
20-54	7.64	6.48	6.19
20-49	7.69	7.55	4.78
20-50	7.92	8.57	6.31
20-69	8.08	6.85	5.71
20-22	8.16	9.73	5.72
20-33	8.35	7.54	5.70
20-60	8.39	9.03	6.42
20-80	8.60	8.45	7.37
20-81	8.89	7.75	4.96
20-23	8.90	7.97	4.63
20-62	8.96	8.40	5.69
20-27	8.97	8.72	6.16
20-34	9.15	10.15	5.26
20-88	9.72	8.23	6.29
20-96	10.02	6.92	7.63
20-39	10.69	10.15	4.77
20-99	11.15	12.10	6.67
20-44	11.36	13.06	6.87
V99-5089	11.57	10.79	--
20-7	11.62	11.86	8.94
20-46	11.77	11.67	7.69
20-58	12.08	11.74	7.89

Table 8: Comparison of raffinose values from F2 families with $F_{2:3}$ data from Blacksburg and Warsaw and F_2 greenhouse data

	Raffinose (% DW) from:		
F_2 plant	Blacksburg	Warsaw	Greenhouse
20-46	0.34	0.44	1.79
20-99	0.35	0.39	1.49
20-58	0.36	0.32	0.86
V99-5089	0.36	0.29	--
20-7	0.37	0.46	0.54
20-44	0.41	0.35	0.71
20-39	0.47	0.78	1.62
20-34	0.49	0.68	2.16
20-62	0.50	0.68	1.80
20-23	0.52	0.95	1.13
20-96	0.52	0.74	1.21
20-33	0.53	0.69	1.20
20-80	0.58	0.77	1.31
20-53	0.62	0.68	1.75
20-60	0.63	0.73	1.23
20-22	0.64	0.57	1.38
20-27	0.64	0.65	2.07
Essex	0.64	0.68	--
20-50	0.65	0.81	1.82
20-88	0.66	0.62	1.62
20-81	0.68	0.66	1.48
20-49	0.70	0.70	2.38
20-84	0.70	0.71	1.61
20-14	0.71	0.80	1.21
20-54	0.73	0.89	1.53
20-48	0.75	0.88	1.22
20-35	0.76	0.84	1.54
20-69	0.76	0.78	1.39

Table 9: Comparison of stachyose values from F2 families with $F_{2:3}$ data from Blacksburg and Warsaw and F_2 greenhouse data

	Stachyose (% DW) from:		
F_2 plant	Blacksburg	Warsaw	Greenhouse
20-58	0.17	0.26	1.06
20-99	0.22	0.33	2.07
20-44	0.26	0.33	0.55
20-7	0.26	0.45	0.35
V99-5089	0.27	0.22	--
20-46	0.31	0.53	2.14
20-96	1.82	4.68	3.43
20-39	1.90	4.06	2.70
20-23	2.19	5.40	2.60
20-62	2.26	3.30	3.81
20-34	2.33	4.39	3.68
20-22	2.52	1.93	2.57
20-33	2.60	3.68	2.64
20-80	2.71	4.09	3.76
20-81	2.93	3.41	3.63
20-27	3.00	3.26	2.93
20-88	3.17	3.06	2.79
20-60	3.49	4.60	3.26
20-50	3.63	5.33	3.68
20-14	3.92	4.70	4.78
20-49	4.00	4.83	3.92
20-48	4.23	5.35	5.29
20-69	4.37	5.08	3.62
Essex	4.44	5.58	--
20-53	4.48	5.13	4.42
20-35	4.52	6.10	3.85
20-84	4.53	5.16	5.32
20-54	4.92	5.13	4.77

plant stress because greenhouse conditions are generally more stressful than field conditions for soybean plants and these results show a trend toward higher raffinosaccharide production in the greenhouse. If this is the case, the reduced levels of sucrose observed in seeds produced in the greenhouse would be explained by the inverse relationship between sucrose and the raffinosaccharides.

The data in Table 6 raise some questions about PI87013, which was assumed to be the source of the low stachyose phenotype in V99-5089. PI87013 was reported to have somewhat reduced stachyose levels by Hymowitz et al. (1972), but it had an average stachyose level of 4.38% in this study which is similar to the normal stachyose parents. Clearly PI87013 is not homogenous for the low stachyose trait.

In each cross, there are fewer low stachyose families than families having high levels of stachyose (Figure 2). The bimodal distribution suggests simple inheritance. The number of individuals in each of the two classes is a good fit to a 3 high:1 low monogenic ratio for V99-5089 x Essex and V99-5089 x V99-6180 but not with PI87013 x MFL-552 (Table 5). The overall distribution for the latter is an acceptable fit to a 15:1 ratio however. A 3:1 ratio suggests that a single diallelic gene is controlling stachyose content while a 15:1 ratio would correspond to control by two diallelic genes.

The only parental cultivar with low stachyose was V99-5089 so it seems reasonable to expect a single gene to be segregating in populations with V99-5089 as a parent. PI87013 and MFL-552, the parents of V99-5089, both have high average stachyose levels and, assuming the same inheritance, their hybrid progeny should produce only progeny with high stachyose levels, clearly that was not the case. It is unlikely that MFL-552 contains a low stachyose gene because this cultivar is used as a check sample for sugar analysis in the Virginia Tech soybean breeding program and there has never been an indication of the presence of a low stachyose allele. Therefore, if there were two genes segregating for stachyose content in PI87013 x MFL-552, both of them likely came from PI87013. If this were the case, PI87013 should exhibit low stachyose, both of these genes would be present in V99-5089, and both crosses with V99-5089 should segregate 15 high:1 low.

In Figure 2 it appears that high stachyose families of V99-5089 x Essex and V99-5089 x V99-6180 have a wider distribution than the high stachyose families of PI87013 x

MFL-552. The families in the high stachyose group of PI87013 x MFL-552 have a higher mean than the high stachyose families of either of the other two populations. This could indicate a narrower genetic base for the PI87013 x MFL-552 cross or an unusually high number of high stachyose individuals resulting from the PI87013 x MFL-552 cross.

Because the families in each cross were derived from more than one F_1 plant, the distribution of high and low stachyose families derived from each F_1 plant was investigated in an attempt to resolve the apparent discrepancies. From Table 5 it is clear that all F_1 plants of V99-5089 x Essex and V99-5089 x V99-6180 produced some low stachyose F_2 families but only two of the F_1 plants of PI87013 x MFL-552 produced low stachyose families while the others produced only high stachyose families. The F_1 plants producing no low stachyose families also have a higher F_1 family mean than the average of the high stachyose F_2 families from F_1 plants 1-1 and 1-3, which produced segregating F_1s. In addition, the families from F_1 plants producing only high stachyose F_2 plants were relatively uniform as indicated by the standard deviation compared to the high stachyose families from F_1 plants producing both high and low stachyose families.

While the number of plants in the non-segregating families is not large enough to rule out the possibility that the F_1 plants were really heterozygous, these observations indicate that the F_1 plants producing non-segregating families were either the result of self-pollinations of a high stachyose line or a cross between two high stachyose lines because all of the progeny have uniformly high stachyose levels. Because all families of population 1 are segregating for hilum color (Table 5) it is reasonable to rule out the possibility that F_1 plants 1-2, 1-4, 1-5, and 1-6 resulted from self-pollination. Also, PI87013, the assumed source of the low stachyose gene, is the female parent of population 1 and self-pollinations on low stachyose plants would result in all low stachyose progeny. This lack of uniformity among F_1 plants indicates that the PI87013 plants used in crossing probably were not uniform for stachyose content. The heterogeneous nature of PI87013 is also supported by the fact that Hymowitz et al. (1972) reported 1.5% stachyose for PI87013 compared to 2.9% average for the group of PIs they evaluated. This is consistent with PI87013 being a mixture of low and high stachyose plants because the stachyose content of our low stachyose plants is much lower than 1 %. It appears that PI87013 plants with the low stachyose phenotype were used to produce $F_1$1-1 and $F_1$1-3 and plants with high

stachyose produced the other F_1 plants. The combined segregation of $F_{2:3}$ families from $F_1$1-1 and $F_1$1-3 show a good fit to a 3 high: 1 low ratio (χ^2=0.4286, p=0.5-0.7). This result, along with the apparent segregation in Populations 2 and 3, make it reasonably clear that a single gene for low stachyose is present in PI87013. Only the families derived from these F_1s will be considered in further discussions of PI87013 x MFL-552.

Segregation of families for stachyose content was a good fit to a 3:1 ratio for all three crosses when the probable off types were eliminated from population 1 (Table 5). This indicates that a single gene has a significant influence on stachyose content of soybean seeds. Similar results were obtained with the individual plants of V99-5089 x Essex (Table 14). All segregating families exhibited a good fit to the 3 high:1 low ratio except one family which had a p value below 0.05. However, the homogeneity test shows the families are segregating homogeneously and the overall distribution is a good fit to a single gene model (Table 14). In addition, the overall fit of the families to a 1 high: 2 segregating: 1 low ratio, as expected in the segregation of a single gene, is good (χ^2=0.1039; p=0.7-0.8).

Because both F_2 and F_3 observations provide good fits to expected ratios, it would be reasonable to conclude that a single gene confers the low stachyose phenotype. It then becomes feasible to identify homozygous and heterozygous genotypes; homozygous low families contain all low stachyose seeds while the homozygous high genotype produces high levels of stachyose in the seed. Observation of average stachyose levels of each family with regard to the proposed genotype (Table 15) shows the homozygous high families to have obviously higher levels of stachyose than the homozygous low families and the heterozygous families appear to have a wider range of stachyose contents than the homozygous groups.

Figure 3 shows the distribution of stachyose in individual plants in the homozygous high, homozygous low, and segregating families of population 2. The families that are homozygous for the high and low stachyose traits all have extreme levels of stachyose (avg= 4.37 and 0.25% respectively) while the segregating families shared between the two homozygous groups (avg=3.3%). If complete dominance were the case, we would expect to see only two distinct classes because the heterozygotes have the same phenotype as the homozygous dominant individuals. On the other hand, if the

Table 10: Segregation of individual plants in $F_{2:3}$ families from V99-5089 x Essex for stachyose content.

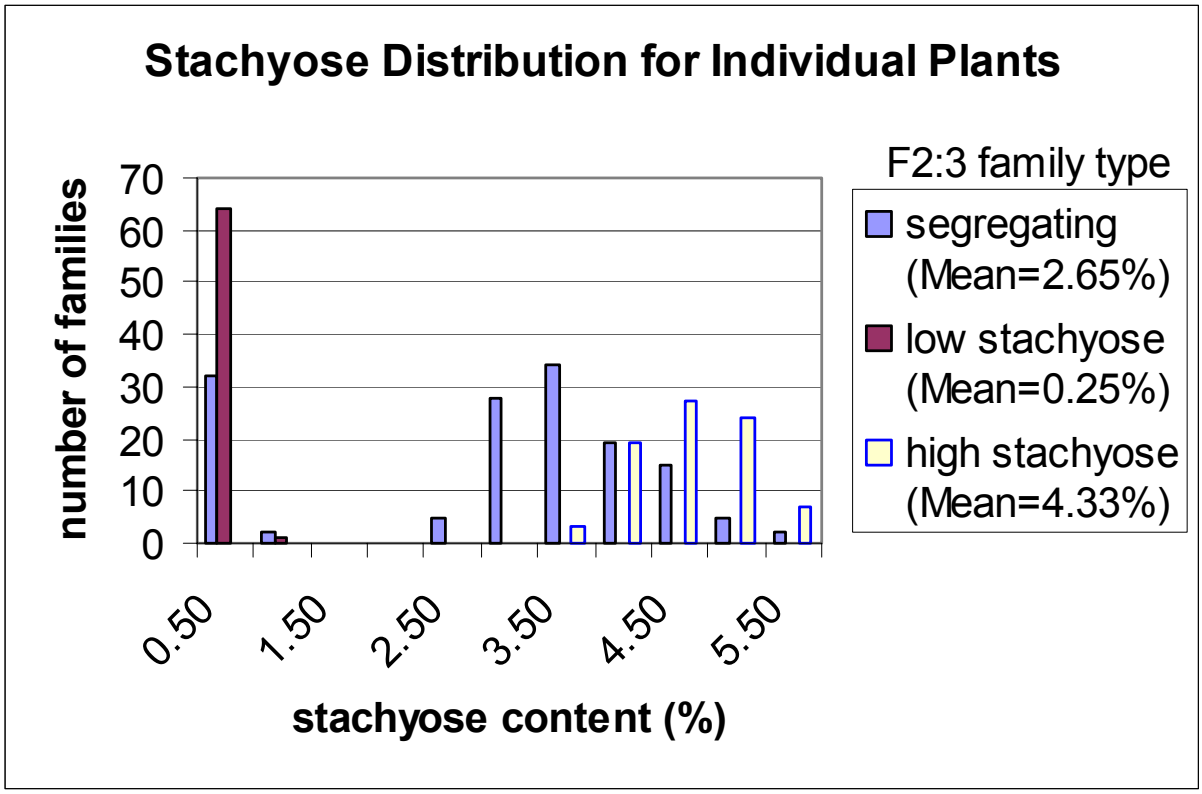
	Number of plants		Heterogeneity test for segregating families		
Entry	High†	Low†	$\chi^2_{3:1}$	df	p
113	0	10	--	--	--
154	0	11	--	--	--
142	0	6	--	--	--
60	0	11	--	--	--
110	0	10	--	--	--
100	0	9	--	--	--
98	0	9	--	--	--
152	5	6	5.121	1	.01-.05
92	4	4	2.667	1	.1-.2
117	8	4	0.444	1	.5-.7
76	8	4	0.444	1	.5-.7
87	6	3	0.333	1	.7-.8
75	8	2	0.133	1	.7-.8
86	10	2	0.444	1	.5-.7
71	8	2	0.133	1	.7-.8
135	7	1	0.677	1	.3-.5
136	9	1	1.200	1	.2-.3
80	9	1	1.200	1	.2-.3
143	8	2	0.133	1	.7-.8
115	10	1	1.485	1	.2-.3
104	8	1	0.926	1	.3-.5
67	11	0	--	--	--
103	12	0	--	--	--
102	10	0	--	--	--
109	7	0	--	--	--
124	12	0	--	--	--
108	8	0	--	--	--
88	11	0	--	--	--
139	9	0	--	--	--
	Total χ^2		**15.332**	**14**	**.3-.5**
	Divergence		**0.085**	**1**	**.7-.8**
	Heterogeneity		**15.247**	**13**	**.2-.3**

† low<1<high

Table 11: Average sucrose, raffinose, and stachyose content of seeds from individual $F_{2:3}$ plants of V99-5089 x Essex

		% based on dry seed wt of:			
Entry	assumed genotype	Sucrose	Raffinose	Stachyose	Total sugar
113	aa	12.08	0.36	0.17	12.61
154	aa	11.15	0.35	0.22	11.72
142	aa	11.56	0.30	0.25	12.10
60	aa	11.62	0.37	0.26	12.25
110	aa	12.08	0.25	0.30	12.63
100	aa	11.77	0.34	0.31	12.42
98	aa	11.36	0.41	0.24	12.01
152	Aa	10.02	0.52	1.82	12.35
92	Aa	10.69	0.47	1.90	13.06
117	Aa	8.96	0.50	2.27	11.72
76	Aa	8.71	0.53	2.29	10.64
87	Aa	9.15	0.49	2.33	11.96
75	Aa	8.16	0.64	2.52	11.32
86	Aa	8.39	0.53	2.54	11.46
71	Aa	8.45	0.60	2.60	11.65
135	Aa	8.60	0.58	2.71	11.89
136	Aa	8.89	0.68	2.94	12.51
80	Aa	8.86	0.63	3.03	12.52
143	Aa	9.72	0.66	3.17	13.56
115	Aa	8.38	0.63	3.49	12.50
104	Aa	7.92	0.65	3.63	12.20
67	AA	6.71	0.71	3.92	11.34
103	AA	7.69	0.70	4.00	12.38
102	AA	6.88	0.75	4.23	11.87
109	AA	7.64	0.73	4.93	13.30
124	AA	8.08	0.76	4.37	13.21
108	AA	6.97	0.62	4.48	12.07
88	AA	7.16	0.76	4.52	12.45
139	AA	6.71	0.71	4.53	11.94
aa average		11.66	0.34	0.25	12.25
AA average		7.23	0.72	4.37	12.32

Figure 3: Distribution of stachyose in individual plants harvested from population 2 at Blacksburg.



alleles for stachyose content were co-dominant, we would expect the heterozygotes to have a mean of 2.31% stachyose which is the average of the high and low stachyose means. However, the average stachyose content of the segregating families was 3.3%. This indicates the gene action to be neither completely dominant nor codominant but rather suggests partial dominance. The means of the high, low, and segregating groups indicate that the high stachyose gene has approximately a 0.5 degree of dominance.

This 'stachyose gene' also has an effect on the other sugars in soybean seeds. Table 15 shows the relationship between the amount of sucrose, raffinose, and stachyose content in the $F_{2:3}$ families that were analyzed as single plants. The homozygous low stachyose families had an average sucrose content of 11.7% on a dry weight basis while the homozygous high families had an average of 7.23%. This indicates an inverse relationship between stachyose and sucrose content in soybean seeds as reported previously (Hymowitz et al., 1972). Raffinose content, on the other hand, appears to have a positive relationship with stachyose content; low stachyose families tend to have lower raffinose content than the high stachyose families. There was little difference in total sugar content between the high and low stachyose groups but the proportion of the individual sugars was different in each case. The average sucrose content of low stachyose families was 4% higher than the high stachyose families while raffinose content was 0.38% higher in the high stachyose group.

Quantitative Genetics Study

Heritability estimates were calculated from variance components for sucrose, raffinose, and stachyose content of individual plants and plots from the V99-5089 x Essex population grown at Blacksburg. Because the segregation of a major gene controlling stachyose content would inflate the heritability estimates, only the homozygous high and homozyous low families were used to calculate heritability estimates (Table 12). Each sub-population was treated separately. Individual plant data made it possible to identify homozygous entries among the bulk harvested entries with reasonable certainty in population 2 but the homozygous high plots did not differ significantly from the heterozygous plots and there was no way to differentiate between them for population 1 or 3, so only population 2 was used for the quantitative study.

Table 12: Variance components and heritability estimates calculated from ANOVAs of individual plants (IP) and plot means of V99-5089 x Essex $F_{2:3}$ families grown at Blacksburg (BB) and Warsaw (W), VA

	Sugar trait	Source of variation	Variance components	V_A	V_E	h^2
Low stachyose						
BB (plot)	suc	entry	0.000	0.000	1.274	0.000
BB (plot)		residual	1.274			
BB (plot)	raf	entry	0.000	0.000	0.005	0.000
BB (plot)		residual	0.005			
BB (plot)	stac	entry	0.004	0.004	0.001	0.886
		residual	0.003			
W (plot)	suc	entry	0.851	0.851	0.325	0.724
W (plot)		residual	0.750			
W (plot)	raf	entry	0.002	0.002	0.002	0.464
W (plot)		residual	0.003			
W (plot)	stac	entry	0.008	0.008	0.005	0.626
W (plot)		residual	0.009			
BB (IP)	suc	entry	0.0441	0.0441	0.873	0.048
BB (IP)		residual	0.8955			
BB (IP)	raf	entry	0	0	0.028	0.000
BB (IP)		residual	0.02755			
BB (IP)	stac	entry	0.001584	0.001584	0.005	0.256
BB (IP)		residual	0.005406			
High stachyose						
BB (plot)	suc	entry	0.09048	0.09048	0.334	0.213
BB (plot)		residual	0.3796			
BB (plot)	raf	entry	0.000521	0.000521	0.004	0.118
BB (plot)		residual	0.004151			
BB (plot)	stac	entry	0.06374	0.06374	0.077	0.452
BB (plot)		residual	0.1092			
W (plot)	suc	entry	0	0	0.726	0.000
W (plot)		residual	0.7262			
W (plot)	raf	entry	0	0	0.011	0.000
W (plot)		residual	0.01139			
W (plot)	stac	entry	0	0	0.429	0.000
W (plot)		residual	0.4292			
BB (IP)	suc	entry	0.2322	0.2322	0.357	0.394
BB (IP)		residual	0.4728			
BB (IP)	raf	entry	0.001218	0.001215	0.008	0.129
BB (IP)		residual	0.008825			
BB (IP)	stac	entry	0.09367	0.09367	0.108	0.463
BB (IP)		residual	0.1553			

The individual plant families show a greater amount of additive variance than the bulk harvested plots for all three sugars in the high stachyose families but the low stachyose families have way more variation from Warsaw than from either the individual plants or bulk harvested plots at Blacksburg. In the case of the bulk harvested families, samples are composites of multiple plants for each family so the actual differences may be masked by averaging plants with high and low amounts of a particular sugar and obtaining intermediate values which are used for calculating variance components. The individual plants, on the other hand, are not subjected to averaging of plants within a family and the variation observed is greater than in the bulk harvested families.

Residual variance would be expected to follow a similar pattern with the bulk harvested plots having less residual variance than the individual plants. As seen on Table 12, the data from this study do not follow this pattern. There is more residual variation present in the bulk harvested plots from high stachyose families than would be expected because nearly equal amounts of residual variance are observed in the high stachyose individual plant families and bulk harvested families. Residual variation includes the block effect on stachyose content, which appears to be greater than expected in the bulk harvested plots. Non- significant block effects were observed for sucrose, raffinose, and stachyose in these plots. It seems unlikely that these block effects are reducing the residual variance to the extent observed but may contribute to the decline. Another factor that may be contributing to the apparent excess of residual variation for the bulk harvested families might be a lack of residual variation among the individual plants. Because individual plants were harvested from plots having the most vigorous plants as well as those that had at least seven plants among the two blocs, it is not unreasonable that these plots might have less than the expected level of residual variation. Thus, comparing the residual variation of the bulk harvested plots to that of the individual plots would result in higher than expected levels of variation. Likewise, heritability estimates of plot means should be higher than heritability estimates based on individual plants, which, as seen on Table 12, is not the case for these data. Also, analyses from Warsaw show a greater level of environmental variation in nearly all cases.

The heritability of sucrose content appears to be affected by the presence of the low stachyose phenotype. In the presence of the major gene controlling stachyose

content, there is very little additive variance remaining. Data from Warsaw show relatively high heritability estimates for sucrose among the low stachyose plots but the individual plants and plots from Blacksburg resulted in low heritability estimates. However, past experience has shown higher levels of heritability for sucrose; broad sense heritabilities calculated for large seeded breeding line tests grown at four locations in Virginia from 1999 to 2001 ranged from 0.58 to 0.91 for sucrose. As seen on Table 14, sucrose content also had a high level of environmental variation (V_E) which is consistent with the idea that this trait is quantitatively inherited and controlled by many genes as previously suggested (Bentsink et al., 2000; Cicek, 2001; Maughan et al., 2000).

As can be seen in Table 12, raffinose has little to no variability within the high or low stachyose group. This is evident in the low additive variance and heritability estimates for raffinose in both the high and low stachyose groups (Table 12). This indicates that little progress would be expected when selecting for raffinose content in the V99-5089 x Essex population. It could be that either the "low stachyose" allele is affecting a step of the RFO pathway prior to raffinose formation such that it confers both low raffinose and low stachyose content at the same time or that there is some sort of feedback inhibition occurring and the low levels of stachyose prevent the formation of raffinose while the higher levels of stachyose stimulate greater production of raffinose.

Stachyose content shows moderate levels of variability even after the effects of the major gene are removed with more environmental variation apparent in the high stachyose group than the low stachyose group. This suggests that the low stachyose phenotype may involve genetic factors influencing the final stachyose content in addition to the major gene for stachyose content.

There is a greater amount of additive variance for both sucrose and stachyose for the high stachyose groups than the low stachyose groups from Blacksburg. This indicates that the minor genetic factors contributing to soluble oligosaccharide content have more effect in the absence of the major gene controlling stachyose content than in its presence. It is interesting to notice that the heritability estimate for stachyose from the low stachyose plots harvested at Blacksburg is very high (h^2=0.886) which means selecting for stachyose should result in rapid improvement, however, due to the low additive

variance present, little progress would be made by selecting for stachyose content among low stachyose families.

Relationship Among Soybean Seed Components

The data obtained in this experiment support those previously reported about the relationship of sucrose, raffinose, and stachyose content in soybean seeds (Geater et al, 2000;Geater and Fehr, 2000;Wilcox and Shibles, 2000; Hymowitz et al., 1972; Hartwig et al., 1997). Significant correlations exist among sucrose, raffinose, and stachyose, but total sugar was not strongly correlated with any of the three individual sugars (Table 13).

The lack of a correlation of the individual sugars with total sugar is probably related to the low level of variation present for total sugar (Table 13) such that means of low and high stachyose groups are similar. This result may be attributed to the inverse relationship between sucrose and the raffinosaccharides; as one increases and the other decreases, the total amount of sugar remains relatively stable. Table 13 shows a highly significant inverse correlation between sucrose and both stachyose and raffinose. There was a highly significant positive correlation between stachyose and raffinose.

Agronomic Trait Comparison

Table 14 shows the means calculated over the high and low stachyose families from V99-5089 x Essex and V99-5089 x V99-6180 for maturity, mature plant height, number of plants per plot, weight of seed harvested, and soluble oligosaccharide content for both locations. No significant differences were found between the high and low stachyose groups for number of plants per plot, maturity, mature plant height, or weight of seed harvested which suggests that seed stachyose content has little effect on agronomic characteristics of soybean plants. The yield of seed per plot was slightly lower for the low stachyose lines of both populations, but when yields are calculated on a per plant basis, the trend was either nullified or reversed. Thus, there is no consistent trend for more or less seed production from plants producing seeds with low stachyose content. Likewise, there was no consistent trend for the low or high stachyose groups or V99-5089 to yield differently from the check cultivar Essex. The lack of significant

Table 13: Phenotypic correlation of sucrose, raffinose, stachyose, and total sugar for 29 family averages of V99-5089 x Essex based on 288 individual observations

	Sucrose	Raffinose	Stachyose
Raffinose	-0.912		
(p)	*(<0.001)*		
Stachyose	-0.944	0.950	
(p)	*(<0.001)*	*(<0.001)*	
Total	0.215	0.091	0.119
(p)	*(0.281)*	*(0.651)*	*(0.554)*

Table 14: Comparison of average characteristics for entries with high and low levels of stachyose from V99-5089 x Essex and V99-5089 x V99-6180 grown at Blacksburg, VA

				% seed dry weight of:							
Pedigree	Loc †	Families‡	Plants per plot	suc	raf	stac	total	maturity§	Plant ht (cm)	Seed yield (g/plot)	Seed yield (g/plant)
V99-5089 x Essex	BB	15 low	4.7	12.0***	0.4***	0.3***	12.7	68	15	66	14
V99-5089 x Essex	BB	15 high	4.8	7.4	0.7	4.5	12.6	67	15	75	16
V99-5089 x Essex	W	15 low	3.2	11.2***	0.4***	0.4***	12.0	65	9	34	11
V99-5089 x Essex	W	15 high	4.2	7.1	0.8	5.1	13.0	61	9	41	10
V99-5089 x V99-6180	BB	6 low	3.3	11.2***	0.5***	0.4***	12.0	68	36	63	19
V99-5089 x V99-6180	BB	6 high	4.3	7.2	0.8	4.5	12.5	64	36	76	18
V99-5089 x V99-6180	W	4 low	2.5	NA	NA	NA	NA	64	19	21	8
V99-5089 x V99-6180	W	4 high	4.4	NA	NA	NA	NA	62	22	37	8
V99-5089	BB	4 low	5.0	11.7	0.3	0.2	12.3	65	17	101	20
V99-5089	W	3 low	5.0	10.8	0.3	0.2	11.3	69	16	39	8
V99-6180	BB	2 high	5.0	5.7	1.0	5.0	11.8	70	15	98	20
V99-6180	W	2 high	4.0	NA	NA	NA	NA	66	9	27	7
Essex	BB	4 high	4.5	6.1	0.6	4.4	11.2	71	16	73	16
Essex	W	4 high	4.3	6.1	0.7	5.5	12.3	61	11	57	13

† BB=Blacksburg; W=Warsaw

‡ low<1<high stachyose content

§ maturity is recorded as the number of days after August 31 that 95% of the pods are brown

*,**,*** different from high mean for same cross at p=.05, .01, and .001 respectively

differences in plant stands between the low and high stachyose groups is in agreement with Bentsink et al. (2000) who reported that seed germinability of *Arabidopsis* was not affected by RFO or sucrose content. However, the trend for lower plant stands in the low stachyose entries in this study indicate a need for further investigation. Overall, these preliminary data are not sufficient to draw any conclusions, but appear to indicate that there are no seriously undesirable agronomic traits associated with the low stachyose phenotype.

Phosphorus Results

A range of 1.2 to 35.9 x 10^{-4} % inorganic phosphorus and 0 to 16.7 x 10^{-4} % phytate phosphorus was observed in the soybean seeds tested. Table 15 shows the ranges found in each stachyose level in two populations and the parents. Little to no relationship was detected between inorganic and phytate phosphorus fractions (Figure 4A). The positive relationship reported by Wilcox et al. (2000) between phytate phosphorus and inorganic phosphorus was not observed in this study. Despite a significant correlation ($r=.29$; $p<0.05$) between phytate and stachyose content, both high and low stachyose plots appear to have both high and low phytate content (Figure 4B). There is a significantly negative relationship ($r=0.88$; $p<0.001$) between inorganic phosphorus content and stachyose content in Figure 4C. It is interesting to note that V99-5089 has both low stachyose and phytate phosphorus levels along with high inorganic phosphorus content. Such lines would likely provide improvements in digestibility. Of the 23 low stachyose samples evaluated, 15 had less the 2 x 10^{-4} g phytate phosphorus per g DW and 12 had greater than 20 x 10^{-4} g inorganic phosphorus per g DW; 7 samples were observed to have less than 1% stachyose, less than 2 x 10^{-4} g phytate phosphorus per g DW and greater than 20 x 10^{-4} g inorganic phosphorus per g DW. However, none of the samples analyzed was superior to V99-5089 in terms of having low stachyose and raffinose along with high inorganic phosphorus.

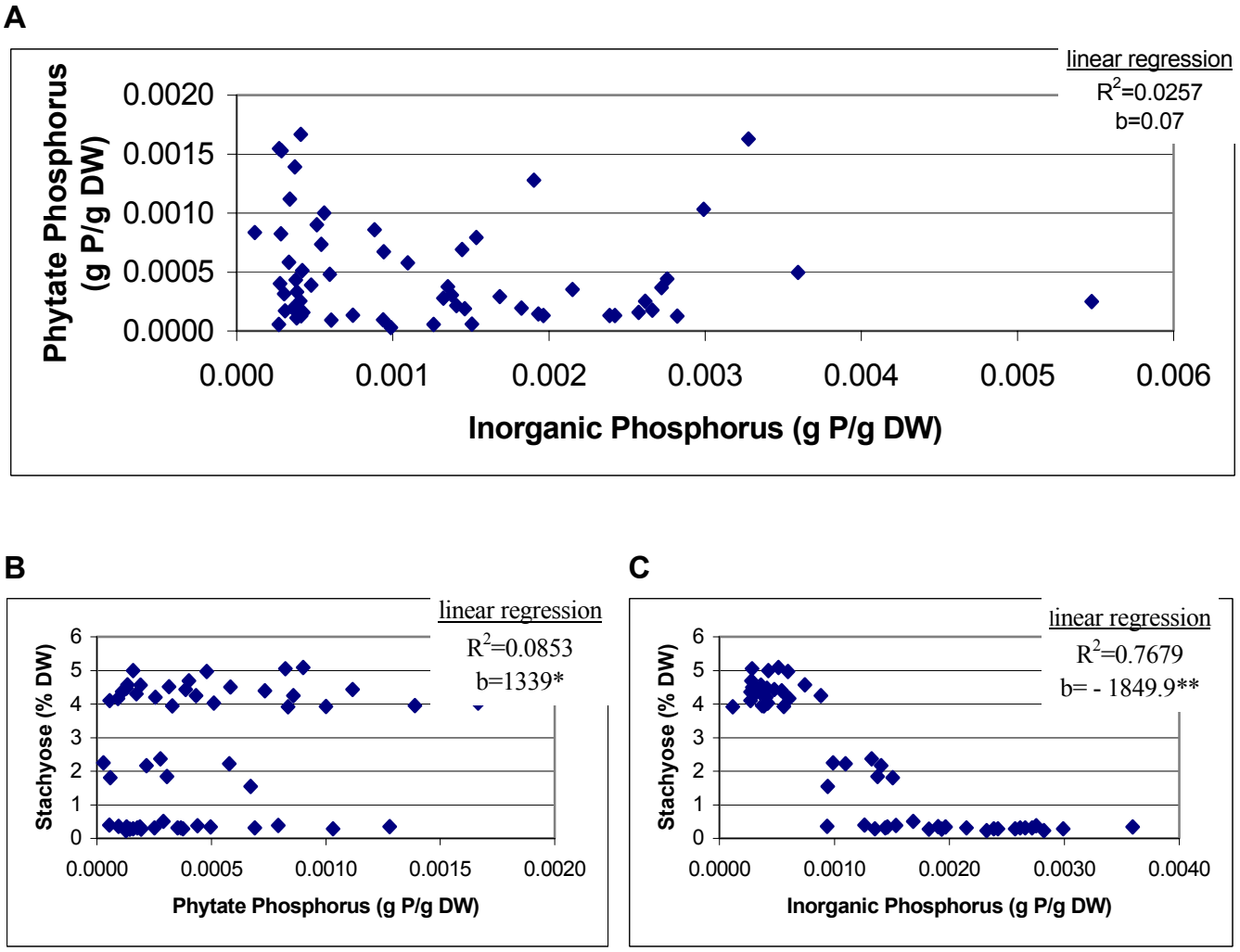
The low stachyose seeds appear to have both high and intermediate levels of inorganic phosphorus while the high stachyose seeds have low inorganic phosphorus and there is essentially no overlap between the two groups. Examining these data separately for high and low stachyose groups had little effect on the overall relationship between

Table 15: Range of phytate phosphorus and inorganic phosphorus measured in lines from crosses 1 and 2 with high, medium (med), and low stachyose levels

Pedigree	No. of samples	stachyose level	inorganic phosphorus†		phytate phosphorus†	
			range	mean	range	mean
V99-5089 x Essex	13	high	2.7-8.8	5	0.9-16.6	6
V99-5089 x Essex	9	med	3.9-15.1	11	0.3-10.0	4
V99-5089 x Essex	14	low	13.5-35.9	23	0.0-12.8	4
V99-5089	4	low	23.9-28.2	26	1.3-1.6	1
Essex	2	high	2.8-4.3	4	1.6-4.0	3
PI87013 x MFL-552	5	high	1.2-4.2	3	5.1-13.9	9
PI87013 x MFL-552	5	low	9.4-18.2	14	0.5-6.9	2
PI87013	2	"low"	2.7-4.1	3	0.6-2.6	2
MFL-552	2	high	3.0-3.8	3	3.2-4.3	4

† g P/g DW x 10^{-4}

Figure 4: Interrelationship of inorganic and phytate phosphorus components with each other (A) and stachyose (B&C) in soybean seeds from high, low, and medium stachyose families of crosses 1 and 2 and their parents



*,** indicates significance at p=0.05 and p=0.001respectively

inorganic phosphorus and phytate phosphorus. Data presented here are only preliminary and further study needs to be done before conclusions can be drawn about the inorganic and phytate phosphorus levels of this material. However, it does point out the possibility that the association between stachyose and phytate contents might not be a direct one.

LITERATURE CITED

Bentsink, L., C. Alonso-Blanco, D. Vreugdenhil, K. Tesnier, S. P.C. Groot, and M. Koornneef. 2000. Genetic Analysis of seed-soluble oligosaccharides in relation of seed storability of arabidopsis. Plant Physiol. 124:1595-1604.

Black, L.T. and J.D. Glover. 1980. A simple and rapid HPLC analysis of sugars in soybeans and factors affecting their standardization. JAOCS, April 1980:143-144.

Black, LT. And E.B. Bagley. 1978. Determination of oligosaccharides in soybeans by high pressure liquid chromatography using an internal standard. JAOC 55:228-232.

Bewley, J.D., F.D. Hempel, S. McCormick, and P. Zambryski. 2000. Reproductive Development. P. 988-1043 *In* B.B. Buchanan, W. Gruissem, and R.L. Jones (ed) Biochemistry and Molecular Biology of Plants, American Society of Plant Physiolosts. Rockville, MD.

Chen, P.S., T.Y. Toribara, and H. Warner. 1956. Microdeterminations of phosphorus. Anal. Chem. 28:1756-1758.

Cicek, M. 2001. Genetic marker analysis of three major carbohydrates in soybean seeds. Ph.D. diss. Virginia Polytechnic Inst. & State Univ., Blacksburg, VA.

Cober, E.R., J.A. Fregeau-Reid, L.N. Pietrzak, A.R. McElroy, and H.D. Voideng. 1997. Genotype and environmental effects on natto soybean quality traits. Crop Sci. 37:1151-1154.

de Lumen, B.O. 1992. Molecular strategies to improve protein quality and reduce flatulence in legumes: a review. Food Structure 11:33-46.

Denbow, D.M., Ravindran, V., Kornegay, E.T., Yi, Z., and R.M. Hulet. 1995. Improving phosphorus availability in soybean meal for broilers by supplemental phytase. Poultry Sci. 74:1831-1842.

Dey, P.M. and R.A. Dixon, Eds. 1985. Biochemistry of storage carbohydrates in green plants. Academic Publishing, Inc.: Orlando, FL.

Geater, C.W. and W.R. Fehr. 2000. Association of total sugar content with other seed traits of diverse soybean cultivars. Crop Sci. 40:1552-1555.

Geater, C.W., W.R. Fehr, and L.A. Wilson. 2000. Association of soybean traits with physical properties of natto. Crop Sci. 40:1529-1534.

Geater, C.W., W.R. Fehr, L.A. Wilson, and J.F. Robyt. 2001. A more rapid method of total sugar analysis for soybean seed. Crop Sci. 41:250-252.

Giangiacomo, R., J.B. Magee, G.S. Birth, and G.G. Dull. 1981. Predicting concentrations of individual sugars in dry mixtures by near-infrared reflectance spectroscopy. J. of Food Sci. 46:531-534.

Gulewicz, P., D. Ciesiolka, J. Frias, C. Vidal-Valverde, S. Frejnagel, K. Trojanowska, and K. Gulewicz. 2000. Simple method of isolation and purification of α-galactosides from legumes. J. Agric. Food Chem. 48:3120-3123.

Harland, B.F. and E.R. Morris. 1995. Phytate: a good or bad food component? Nutrition Res. 15:733-754.

Harland, B.F. and D. Oberleas. 1977. A modified method for phytate analysis using an ion-exchange procedure: application to textured vegetable proteins. Cereal Chem. 54: 827-832.

Hartwig, E.E., T.M. Kuo, and M.M. Kenty. 1997. Seed protein and its relationship to soluble sugars in soybean. Crop Sci. 37:770-773.

Hortin, A.E., G. Oduho, Y. Han, P.J. Bechtel, and D.H. Baker. 1993. Bioavailability of zinc in ground beef. J. Anim. Sci. 71:119-123.

Hymowitz, T. and F.I. Collins. 1974. Variability of sugar content in seed of *Glycine max* (L.) Merrill and *G. soja* Sieb. and Zucc. Agron. J. 66:239-240.

Hymowitz, T., F.I. Collins, J. Panczner, and W.M. Walker. 1972. Relationship between the content of oil, protein, and sugar in soybean seed. Agron. J. 64:613-616.

Johansen, H.N., V.Glitso, and K.E.B. Knudsen. 1996. Influence of extraction solvent and temperature on the quantitative determination of oligosaccharides from plant materials by high-performance liquid chromatography. J. Agric. Food Chem. 44:1470-1474.

Jones, D.A., M.S. DuPont, M.J. Ambrose, J. Frias, and C.L. Hedley. 1999. The discovery of compositional variation for the raffinose family of oligosaccharides in pea seeds. Seed Sci. Res. 9:305-310.

Jood, A. U. Mehta, and R. Singh. 1986. Effect of processing on available carbohydrates in legumes. J. Agric. Food Chem. 34:417-420.

Kearsey, M.J. and H.S. Pooni. 1996. The Genetical Analysis of Quantitative Traits. Chapter 4: Selfing and full-sib mating. Chapman & Hall.

Kennedy, I.R., O.D. Mwandemele, and K.S. McWhirter. 1985. Estimation of sucrose, raffinose and stachyose in soybean seeds. Food Chem. 17:85-93.

Kuo, T.M., J.F. VanMiddlesworth, and W.J. Wolf. 1988. Content of raffinose oligosaccharides and sucrose in various plant seeds. J. Agric. Food Chem. 36:32-36.

Larson, S.R. and V. Raboy. 1999. Linkage mapping of maize and barley *myo*-inositol 1-phosphate synthase DNA sequences: correspondence with a *low phytic acid* mutation. Theor. Appl. Genet. 99:27-36.

Lei, X, Ku, P.K., Miller, E.R., Ullrey, D.E., and M.T. Yokeoama. Supplemental microbial phytase improves bioavailability of dietary zinc to weanling pigs. J Nutr 123:1117-1123.

Lowell, C.A. and T.M. Kuo. 1989. Oligosaccharide metabolism and accumulation in developing soybean seeds. Crop Sci. 29:459-465.

Maughan, P.J., M.A. Saghai Maroof, and G.R. Buss. 2000. Identification and quantitative trait loci controlling sucrose content in soybean (*Glycine max*). Mol Breeding 6:105-111.

Morris, E.R., Ellis, R., Steele, P., and P.B. Moser. 1988. Mineral balance of adult men consuming whole or dephytinized wheat bran. Nutr Res 8:445-458.

Openshaw, S.J. and H.H. Hadley. 1978. Maternal effects on sugar content in soybean seeds. Crop Sci. 18:581-584.

Openshaw, S.J. and H.H. Hadley. 1981. Selection to modify sugar content of soybean seeds. Crop Sci. 21:805-808.

Openshaw, S.J., T. Hymowitz, and H.H. Hadley. 1977. Distribution of sugars in the soybean embryo. Crop Sci. 17:975-977.

Parsons, C.M., Zhang, Y., and M. Araba. 2000. Nutritional evaluation of soybean meals varying in oligosaccharide content. Poultry Sci. 79(8):1127-1131.

Peterbauer, T. and A. Richter. 2001. Bichemistry and physiology of raffinose family oligosaccharides and galactosyl cyclitols in seeds. Seed Sci. Res. 11:185-197.

Radcliffe, J.S. 1997. Quantifying the effects of microbial phytase and diet acidity on Ca and P utilization by weanling pigs. M.S. thesis, Virginia Polytechnic Inst. & State Univ., Blacksburg, VA.

Raboy, V., D.B. Dickonson, and M.G. Neuffer. 1990. Survey of maize kernel mutants for variation in phytic acid. Maydica 35:383-390.

Raboy, V., Gerbasi, P.F., Young, K.A., Stoneberg, S.D., Pickett, S.G., Bauman, A.T., Murthy, P.P.N., Sheridan, W.F., and D.S. Ertl. 2000. Origin and seed phenotype of maize *low phytic acid* 1-1 and *low phytic acid* 2-1. Plant Physiol. 124:355-368.

Raboy,V. 2001. Seeds for a better future: 'low phytate' grains help to overcome malnutrition and reduce pollution. Plant Sci. 6(10):458-462.

Raboy, V., Young, K.A., Dorsch, J.A., and A. Cook. 2001. Genetics and breeding of seed phosphorus and phytic acid. J. Plant Physiol. 158:489-497.

Rassmussen, S.K. and F. Hatzack. 1998. Identification of two low-phytate barley (*Hordeum vulgare* L.) grain mutants by TLC and genetic analysis. Hereditas 129:107-112.

Rufty, T.W. Jr., P.S. Kerr, and S.C. Huber. 1983. Characterization of diurnal changes in activities of enzymes involved in sucrose biosynthesis. Plant Physiol. 73:428-433.

Slominski, B.A., Campbell, L.D., and W. Guenter. 1994. Oligosaccharides in canola meal and their effect on nonstarch polysaccharide digestibility and true metabolizable energy in poultry. Poultry Sci. 73:156-162.

Tweary, H.K. and H.G. Muller. 1991. The fate of some oligosaccharides during the preparation of wari, an Indian fermented food. Food Chem 43:107-111.

van Kempen, T.A.T.G., Kim, I.B., Jansman, A.J.M., Verstegen, M.W.A., Hancock, J.D., Lee, D.J., Gabert, V.M., Albin, D.M., Fahey, G.C. Jr., Grieshop, C.M., and D. Mahan. 2002. Regional and processor variation in the ileal digestible amino acid content of soybean meals measured in growing swine. J. Anim. Sci. 80:429-439.

Wilcox, J.R., G.S. Premachandra, K.A. Young, and V. Raboy. 2000. Isolation of high seed inorganic P, low-phytate soybean mutants. Crop Sci. 40:1601-1605.

Wilcox, J.R. and R.M. Shibles. 2001. Interrelationships among seed quality attributes in soybean. Crop Sci. 41:11-14.

Wolf, R.B., J.F. Cavins, R Kleiman, and L.T. Black. 1982. Effect of temperature on soybean seed constituents: oil, protein, moisture, fatty acids, amino acids, and sugars. JAOCS 59:230-232.

Yaklich, R.W. 1985. Effect of aging on soluble oligosaccharide content in soybean seeds. Crop Sci. 25:701-704.

Yazdi-Samadi, B. R.W. Rinne, and R.D. Seif. 1977. Components of developing soybean seeds: oil, protein, sugars, organic acids, and amino acids. J. Agron. 69:481-486.

VITA

Melissa Rose Huhn was born on October 30, 1977 to Donald and Patricia Huhn in Litchfield, Minnesota. Melissa grew up on a small farm outside of Darwin, MN where she lived with her parents and two sisters. She graduated from Litchfield Senior High School in 1996 and received a B.S. in Science in Agriculture with an emphasis on Agronomy from the University of Minnesota, Twin Cities campus in May 2000. She worked at Top Farm Hybrids in Cokato, MN during the summers while she was in junior and senior high school. During high school she was also the bassoon player for the high school concert band and an active member of the Litchfield FFA chapter; she receive the state degree and was a national finalist in her proficiency area in 1996. At the University of Minnesota Melissa became involved in numerous student organizations including Beta of Clovia Sorority, Gopher Crops and Soils Club, and Collegiate Agri-Women. She also worked on the Oat Research Project and at Minnesota Crop Improvement Association while she was a student at the University of Minnesota. A highlight of her undergraduate career was the 4 ½ months spent in South America as she conducted the research for her undergraduate thesis at INIA at Colonia del Sacramento, Uruguay. Upon graduation in May 2000 she worked in a molecular marker lab for Novartis at Northfield, MN for the summer before returning to work on the University of Minnesota Oat Research Project for one semester before beginning her graduate work in January 2001. Melissa accepted a GRA position at Virginia Polytechnic Institute and State University beginning in January 2001 and received her Master of Science degree in Crop and Soil Environmental Sciences after the second summer session of 2003. While at Virginia Tech, Melissa became involved in several student organizations such as the Graduate Honor Society and the Graduate Student Assembly for which she served as the Vice President of Programs for the 2002-2003 academic year. She plans to begin her PhD study at North Dakota State University in the fall of 2003.