

**OPTIMIZATION OF SOYBEAN CARBOHYDRATE PROFILES AND PROFIT  
POTENTIAL USING VARIABLE PLANTING DATES AND GENETIC  
BACKGROUNDS**

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Master of Science

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by

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The undersigned, appointed by the dean of the Graduate School,

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**OPTIMIZATION OF SOYBEAN CARBOHYDRATE PROFILES AND PROFIT  
POTENTIAL USING VARIABLE PLANTING DATES AND GENETIC  
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## **Introduction**

Soybean [*Glycine max* (L). Merrill] is the most important oilseed crop produced and consumed in the world (Wilcox, 2004), and one of the most important economic crops grown in the United States. Soybeans, on a dry weight basis, consist of 40% protein, 20% oil, 35% carbohydrates, and 5% ash. (Hymowitz and Collins, 1974). Soybean meal is a vital component of livestock feed, particularly for monogastric animals, due to its high protein content (~40%) and for being an excellent source of dietary fiber. In 2015, over 30 million metric tons of soybean meal were used for livestock feed, with poultry and swine using 16.3 (54%) and 6.9 (23%) million metric tons, respectively (ASA, 2016). The three major soluble carbohydrates in soybean include sucrose, raffinose, and stachyose (Karr-Lilienthal et al., 2005); (Liu, 1997). The concentration of the soluble carbohydrates can play a role in determining the usefulness of soybean meal. Of the three major components, only sucrose is nutritionally beneficial and easily digested to be used as metabolizable energy for monogastric animals. Alternatively, the raffinose family of oligosaccharides (RFOs), primarily comprised of raffinose and stachyose, are considered as anti-nutritional factors in soybean meal because they are unable to be digested by monogastric animals due to the lack of the enzyme  $\alpha$ -galactosidase in the stomach and small intestine (Sebastian, 2000). The poor digestion of RFOs can lead to a reduction in metabolizable energy, ultimately resulting in poor weight gain and substantial loss in market value in livestock production operations (Rackis, 1974); (Zuo et al., 1996). As soybean seeds are processed into soybean meal, several steps of soaking, heating, and pressure treatments are used. These processing steps decrease both RFO and sucrose concentrations, but still leave detrimental amounts

of RFOs in the resulting soybean meal (Leske and Coon, 1999). More sophisticated processing and extraction methods are available to reduce RFOs, but they are currently not economically feasible for use in large scale livestock feed production systems. Thus, utilizing genetic and environmental controls to reduce RFOs in soybean seeds is desirable for producing high sucrose, low RFO soybean meal to be used in livestock feed.

Deak et al. (2006) confirmed that genetic control offers a solution to removing undesirable RFOs in soybean. A soybean plant introduction, PI200508, was shown to have reduced levels of RFOs and increased levels of sucrose, which was found to be due to reduced raffinose synthase activity controlled by the mutant allele *stc1* (Sebastian, 2000). Soybean raffinose synthase activity has been studied by Hitz et al. (2002) and Dierking and Bilyeu (2008). These studies have shown that the soybean genome contains at least two expressed genes similar to other characterized raffinose synthases in other plants, raffinose synthase 2 (RS2) and raffinose synthase 3 (RS3), respectively. The novel allele of RS2 from PI200508 has been shown to completely associate with the low RFO phenotype. RS3 is also thought to contribute to the low RFO phenotype, but to a lesser degree as compared to the RS2 allele (Dierking and Bilyeu, 2008). The soybean line LR33 was shown to have reduced levels of RFOs and phytic acid, as well as an increase in sucrose, due to a novel allele of the myo-inositol phosphate-1 synthase gene (MIPS1), which plays an important role in the biosynthesis of raffinose and stachyose (Hitz et al., 2002) (Kerr et al., 2003).

Planting date is a management practice that can affect soybean growth and development, yield, and composition. The environment in which a crop is grown can be manipulated by changing the planting date, and this can alter the range of temperatures,

soil moisture levels, and day lengths that a crop is exposed to. Previous research has shown that for genotypes of the same maturity group, the later that soybeans are planted, the more likely the seed-fill period will take place in cooler temperatures than earlier planted soybeans, which can significantly affect seed composition. Previous research has shown that delaying soybean planting led to significant increases in sucrose concentrations (Bellaloui, 2011); (Jaureguy et al., 2013). Hagely et al. (2013) evaluated soybean lines with different combinations of alleles of the RS2 gene in one location. This study indicated variability between carbohydrate profiles of different genotypes, but showed that each genotypic class had a distinct carbohydrate profile. Another study, conducted by Bilyeu and Wiebold (2016), evaluated soybean lines with different combinations of mutant RS2 and RS3 alleles at different planting dates. This study showed significant increases in sucrose concentrations with later planting dates, and suggested that higher sucrose concentrations are produced with cooler temperatures during the seed-fill period.

For livestock producers, feed rations are specifically formulated to specific nutrient requirements on a least-cost basis. Soybean meal with high sucrose and low RFO concentrations has the potential to increase metabolizable energy by upwards of seven to nine percent (Parsons et al., 2000), and industry participants would be willing to pay a premium for feed that increases metabolizable energy. Since soybean meal provides a source of protein to monogastric animals, it could replace animal-derived protein sources that are currently supplemented due to soybean meal being significantly cheaper. It has been estimated that the potential premium that soybean producers could gain from growing high sucrose/low RFO soybean meal would range from \$0.50 to \$1.00 per

bushel (Parcell et al., 2014; Shivley and Parcell, 2014), in an identity preserved system. Since most premiums are paid on a per bushel basis, producers must take into account the ability of a cultivar to yield well within their selected environment in order to maximize profits gained from growing soybeans for a specialty market in aims of receiving a premium.

The objectives of this study are to expand upon previous research investigating the environmental stability of soybean lines with different sources of genetic variation for carbohydrate profiles. These include combinations of mutant RS2 and RS3 alleles, and a mutant MIPS1 allele across maturity groups III and IV at three locations in Missouri during 2015 and 2016. A secondary objective of this study is to determine the economic impact of utilizing soybean genotypes with a modified carbohydrate profile consisting of high sucrose and low RFO concentrations. Soybean producers in the United States are currently able to receive a premium on top of market value for specialty soybean types (e.g. high sucrose/low RFOs, high oleic, high protein, etc.), and it is thought that soybeans with an improved carbohydrate profile consisting of high sucrose and low RFOs could receive a premium in the United States.

These results will provide useful information to producers aiming to maximize sucrose accumulations and minimize RFO concentrations in Missouri in order to receive premiums on top of market value to increase profit potential.

# **Chapter I**

## **Literature Review**

## **Economic Value of Soybean**

Soybean [*Glycine max* (L). Merrill] is the most important oilseed crop produced and consumed in the world (Wilcox, 2004), and one of the leading economic crops in the United States. The three major producing countries are the United States, responsible for 33% of world production with 107 million metric tons, followed by Brazil with 31% and 100 million metric tons, and Argentina with 18% and 59 million metric tons (ASA, 2016).

In the United States, soybean is the second largest crop grown, behind corn, with 33.4 million hectares planted in 2015. In 2015, Missouri was ninth in hectares planted (2.24 million) and ninth in production (4.9 million metric tons) (ASA, 2016).

Soybeans can be grown for numerous different end uses, including: oil products, whole-bean products and meal products (Smith and Huyser, 1987). In 2015, soybean oil accounted for 61% of the world oilseed production and 68% of the world protein meal consumption. Soybean meal is also a vital component of livestock feed, due in part to its high protein content (~47%) and for being an excellent source of dietary fiber (ASA, 2016).

## **Soybean History**

Soybean was first domesticated in northeastern China in the 11<sup>th</sup> century B.C. At that time, it was grown primarily for the seeds to be used as fresh, fermented and dried food products (Gibson and Benson, 2005). It was not until the late 1700's that soybean was introduced in the United States. During that time, Samuel Bowen, a seaman with the East India Company, brought soybeans to Savannah, Georgia from China and requested

that Surveyor General Henry Yonge grow them on his farm. By 1766, Bowen also began growing soybeans at his nearby plantation in Thunderbolt (Hymowitz and Shurtleff, 2005).

It was not until the mid-1800's that soybean was introduced to the Midwestern United States. Throughout most of the 1800's, soybean was primarily grown as a forage crop for hay, or plowed under as a green manure crop to mend soil structure and fertility (Hymowitz, 1990).

Prior to World War II, the United States imported over 40% of its edible fats and oils (Gibson and Benson, 2005). By the mid-1900's, the United States soybean crop had grown to include over 2 million hectares, and the United States became a large exporter of soybean and soybean products. Today, soybeans are grown in 31 U.S. states, making it the second largest cash crop in the United States, behind corn.

## **Soybean Seed Composition**

Hymowitz and Collins (1974) reported that, generally, on a dry weight basis, the most important soybean seed composition constituents are protein (40%), oil (20%), carbohydrates (35%) and ash (5%).

*Protein:* Soybean is an important source of protein, accounting for 68% of the world's protein meal consumption in 2015 (ASA, 2016). Genetic variation for this trait has been shown to range from 34% to 57%, on a dry weight basis. The average value for seed protein content of the accessions in the USDA germplasm collection is 42% (Wilson, 2004).

Soybean proteins are classified as metabolic and storage proteins, with storage proteins accounting for up to 80% of the total protein content (Murphy, 2008). These proteins are synthesized during seed development and are then stored as protein bodies in mature soybean seeds. The two main types of storage proteins are glycinin (11S) and  $\beta$ -conglycinin (7S) (Hill and Breidenbach, 1974). The 11S-to-7S protein ratio is important for the production of soyfoods, and for when soybeans are used as gelling agents, as this ratio influences the strengths of gels made of soybean protein (Yagasaki et al., 2000).

Soybeans are also a good source of essential amino acids, including: cysteine, histidine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine and valine (Zarkadas et al., 1993). However, soybean protein is generally low in the sulfur-containing amino acids, methionine, cysteine and threonine (Liu, 1997). Glutamic acid has been shown as the amino acid with the highest concentration in soybean (Zarkadas et al., 1993). Glutamic acid and aspartic acid combined, known as acidic amino acids, account for around 25% of the total amino acids. The basic and hydrophobic side amino acids: lysine, arginine, histidine, glycine, alanine, valine leucine and isoleucine, account for around 20% of total amino acids. The aromatic amino acids: phenylalanine, tyrosine and tryptophan, account for around 9% of the amino acid residues in soy proteins. Soybean protein is considered high quality due in part to it having a protein digestibility-correlated amino acid score (PDCAAS) close to 1 (Liu, 2005).

Soy protein isolates are the most highly refined soybean proteins, containing over 90% protein, 0.5% fat, 4.5% ash and 0.3% total carbohydrates on a dry weight basis (Remondetto, 2001) (Kolar, 1985). These isolates are mostly free of unwanted characteristics, including: odor, flavor, color, anti-nutritional factors and flatulence.

Additionally, the high protein concentration provides maximum formulation flexibility when the isolates are integrated into food products (Berk, 1992).

Soybean seed protein content has also been shown to be correlated with other seed composition traits. Previous research has indicated a positive correlation between protein and stachyose content (Hymowitz, 1972) (Geater and Fehr, 2000), while protein was negatively correlated with total sugar content (Jaureguy et al., 2013) (Hymowitz, 1972) (Krober, 1962) and with oil content (Hymowitz, 1972).

*Oil:* In 2015, soybean accounted for 61% of the world's oilseed production, making it the leading oilseed crop in the world (ASA, 2016). The oil, or lipid, component of soybean seed represents ~20% of the total seed weight and is mostly found in the cotyledons (Gerde and White, 2008). Genetic variation for this trait has been shown to range from 8 to 28%, with the average seed oil content in the USDA soybean germplasm collection being 20% (Wilson, 2004).

The lipid component of soybean has multiple functions, such as inclusion in membranes, energy reserves and as a solvent medium. Multiple fatty acids are present in soybeans, including palmitic, stearic, oleic, linoleic and linolenic acid (Gerde and White, 2008). On average, regular soybean oil contains around 11% palmitic (C16:0), 4% stearic (C18:0), 24% oleic (C18:1), 53% linoleic (C18:2) and 7% linolenic acid (C18:3) (Liu, 1997). The first number of the fatty acid abbreviations indicates the length of the fatty acid in number of carbon atoms, while the second number indicates the amount of double carbon-carbon (unsaturated) bonds in the molecule (Wilson, 2004).

Breeding efforts for high oleic and low linolenic acid soybean genotypes have received increased attention in recent years. This fatty acid profile would make soybean

oil healthier due to having a higher concentration of monounsaturated fats and a lower concentration of polyunsaturated fats, along with improving the oxidative and heat stability of the oil. This would also result in low or no trans-fat oil, which provides numerous health benefits for consumption by humans.

*Carbohydrates:* Carbohydrates are the second most abundant component in soybean seed after protein. The carbohydrate component of soybean seed accounts for around 30 to 35% of the seed weight, on a dry-weight basis, and can be higher in the meal (Middelbos, 2008).

Carbohydrates in soybean have two key roles: providing cell structure in the plant, and providing metabolizable energy in soybean meal for nutrition. In soybeans, carbohydrates are comprised of two classes: structural and non-structural carbohydrates (Middelbos, 2008). Structural carbohydrates, jointly referred to as dietary fiber, contain the cell wall polysaccharides, non-cellulose polysaccharides and structural non-polysaccharides such as lignin. These carbohydrates are generally insoluble and cannot be readily digested. (Middelbos, 2008).

The major soluble, or non-structural, carbohydrates consist of three major oligosaccharides: sucrose [35- 55 mg per gram of seed ( $\text{mg g}^{-1}$ )], raffinose (11-16  $\text{mg g}^{-1}$ ) and stachyose (29-36  $\text{mg g}^{-1}$ ) (Karr-Lilienthal et al., 2005); (Liu, 1997). Fructose, glucose and galactose are also minor constituents of the carbohydrate profile in soybean, accounting for less than 1% of the soluble portion (Hymowitz, 1972). Genetic variation has been reported for the various sugar components in the USDA soybean germplasm collection. Seed glucose and fructose contents range from around 0.1 to 2.3%, sucrose

ranges from around 1.5 to 10.2%, stachyose from around 1.4 to 6.7% and raffinose from around 0.1 to 2.1% (Hymowitz, 1972); (Hou et al., 2009).

The concentration of each sugar component can play a role in determining the utility of soybean meal and soyfoods. For example, of the three oligosaccharides, only sucrose is thought to be nutritionally valuable and able to be readily digested by monogastric animals. High sucrose and low stachyose can also improve the nutritional value of livestock feed, as well as improve the flavor and digestibility of soyfoods (Mebrahtu and Devine, 2009).

Additionally, sucrose plays a key role during germination by providing energy to the growing plant (Gardner et al., 2003). It was once thought that raffinose and stachyose were important in the germination process, but a study conducted by Dierking and Bilyeu (2009b) has shown that RFOs are not required for sufficient germination. However, raffinose and stachyose are thought to be involved in seed desiccation resistance (Obendorf, 1997) and serve as transport carbohydrates in the phloem (McCaskill and Turgeon, 2007).

## **Anti-Nutritional Factors in Soybean**

In addition to the many beneficial health factors associated with soybean meal and soyfoods, soybean seeds also contain some anti-nutritional factors.

The raffinose family oligosaccharides (RFOs), primarily comprised of raffinose and stachyose, are considered as anti-nutritional factors in soybean meal. The absence of the enzyme  $\alpha$ -galactosidase in the stomach and small intestine of monogastric animals does not allow for the complete digestion of RFOs (Sebastian, 2000). This specific

enzyme conditions the breakdown of raffinose and stachyose into sucrose and galactose, both of which are more easily digestible by monogastric animals. As a result, the poor digestion of RFOs leads to a decrease in metabolizable energy, as well as increases in flatulence and diarrhea, which can ultimately result in poor weight gain and substantial loss in market value for livestock producers (Rackis, 1974); (Zuo et al., 1996). These negative effects highlight the necessity of removing RFOs from soybean meal and other soyfood products for both human and livestock consumption.

Studies have also reported that the nutritive value of soybean meal can be affected by RFOs. Coon et al. (1990) reported that the removal of unwanted RFOs from soybean meal increased metabolizable energy by as much as 25% and also increased polysaccharide digestibility. The effects of RFOs have been studied in various animal diets, including: chickens (Parsons et al., 2000), dogs (Zuo et al., 1996) and pigs (Smiricky et al., 2002). In addition to presenting anti-nutritional effects such as flatulence and diarrhea, the presence of raffinose and stachyose in soybean meal can affect the uptake of other nutrients (Zuo et al., 1996). Reducing RFOs in soybean meal has the potential to greatly increase nutrient availability and metabolizable energy for monogastric animals.

## **Sources of Variation in Soybean RFOs**

*Raffinose synthase:* Raffinose biosynthesis is mediated by raffinose synthase, an enzyme that belongs to a group of hydrolase family enzymes that perform a galactosyl transfer from galactinol to sucrose. This transfer produces raffinose, with *myo*-inositol formed as a by-product (Dierking and Bilyeu, 2008).

Deak et al. (2006) confirmed that genetic control offers a solution to removing unwanted RFOs in soybean. LR28, also known as PI200508, has been identified as valuable germplasm with reduced levels of raffinose and stachyose and increased sucrose, which was found to be a result from reduced raffinose synthase activity (Sebastian, 2000). This study also discovered that the reduction in RFOs and increase in sucrose was controlled by the recessive allele *stc1a*. A later study conducted by Neus et al. (2005) evaluated the influence of this allele on agronomic and seed traits, with the results showing comparable performance to conventional varieties.

Soybean raffinose synthase activity has also been studied by Hitz et al. (2002) and Dierking and Bilyeu (2008). Previous studies have shown that the soybean genome contains at least two expressed genes similar to other characterized raffinose synthases in other plants, raffinose synthase 2 (RS2) and raffinose synthase 3 (RS3), respectively. A novel allele of RS2 from PI200508 has been shown to completely associate with the low RFO phenotype. RS3 is also thought to contribute to the low RFO phenotype, but to a smaller degree when compared to the RS2 allele (Dierking and Bilyeu, 2008).

Dierking and Bilyeu (2008) investigated the genetic basis of the *stc1a* allele in PI200508, and showed that the low RFO phenotype was caused by a three base pair deletion. This resulted in the deletion of a tryptophan in a highly conserved region of the RS2 protein. A strong association between this variant rs2 allele and the low RFO phenotype was confirmed through a molecular marker assay. Dierking and Bilyeu (2009a) further confirmed these results with the discovery of a mutant identified through Targeting Induced Local Lesions IN Genomes (TILLING). This mutant contained a

missense mutation in the coding sequence of RS2, and also showed a low RFO phenotype.

*Stachyose synthase*: Similar to raffinose synthase, stachyose is formed by the stachyose synthase, which combines raffinose and galactinol to produce stachyose. Until recently, there has been little indication in the literature of stachyose synthase in soybean. Studies by (Peterbauer et al., 1999) and (Peterbauer and Richter, 2001) have shown that stachyose synthase exists in adzuki bean. This stachyose synthase and other similar candidate stachyose synthase sequences are similar to known raffinose synthase sequences (Peterbauer et al., 1999). Until recently, this was the only stachyose synthase enzyme that had been characterized.

Qiu et al. (2015) recently identified a 33-bp deletion mutant in the putative stachyose synthase (STS) gene of PI603176A that controls reduced stachyose content in soybean. The mutant *sts* allele interrupted the conversion of raffinose to stachyose, which was shown to cause a 90% reduction of stachyose and the accumulation of raffinose content in soybean seed (Qiu et al., 2015). The *sts* genotype was shown to always be associated with the low stachyose phenotype (0.5%), significantly lower than the wild type (5%). However, this was also associated with a high raffinose content (4.5%), retaining regular RFO levels overall. While the ideal raffinose/stachyose content in livestock meal has not been well determined, this *sts* allele can prove beneficial when combined with other RS2/RS3 alleles in order to achieve a more beneficial combination of raffinose and stachyose.

*Myo-inositol 3-phosphate synthase (MIPS)*: In soybean, *myo*-inositol is responsible for numerous biological functions including signal transduction, stress

response, cell-wall biogenesis, growth regulation and phosphate biosynthesis (Irvine and Schell, 2001). The precursor for all inositol-containing compounds in the cell, *myo*-inositol, is biosynthesized by the *D-my*o-inositol-3-phosphate synthase (MIPS). This enzyme converts glucose-6- phosphate to *D-my*o-inositol-3-phosphate (Loewus and Loewus, 1983). MIPS can be valuable to plant breeders, due to phytic acid being considered an anti-nutritional component of soybean meal, and also due to *myo*-inositol being the precursor for galactinol use in the biosynthesis of both raffinose and stachyose.

Phytic acid is the primary storage component of seed phosphorus and can account for up to 2% of the soybean seed dry weight (Raboy et al., 1984). Phytic acid is considered an anti-nutritional factor in soybean meal due to the inability of monogastric animals to fully metabolize phytate, and thus, identifying mutants within the MIPS gene family is of high interest (Raboy, 2002). One mutant line, LR33, is characterized by a 50% reduction in seed phytic acid levels as well as having elevated sucrose and a reduction in both raffinose and stachyose. This phenotype is due to a missense mutation in the MIPS1 gene (Hitz et al., 2002); (Kerr et al., 2003). Additional MIPS mutants have also been identified in soybean having a 66.6% increase in available phosphorus and a 46.3% decrease in seed phytic acid levels (Yuan et al., 2007).

## **Planting Date Effects**

Planting date is an important factor that can affect soybean growth and development, seed yield, and seed composition. Although producers cannot control many of the environmental factors that can influence these traits, changing the planting date is a

management practice that alters the environment in which the crop is grown, and can ultimately influence the end product.

Traditionally, the core objective of growing a crop is to maximize yield in order to obtain the highest profit. However, in the future seed quality and composition may become equally important in the case of specialty soybean genotypes. Breeding efforts for genotypes containing unique compositional traits (e.g. high oleic/low linolenic, high protein, high sucrose/low RFOs, etc.) have become more widespread in recent years, thus increasing the need to understand how these unique genotypes best respond to different environmental factors. Because genotypes respond differently to the environment and other management practices, further information is needed to determine the best practices to utilize when growing specialty soybeans.

Photoperiodism is the key physiological process that affects reproduction and triggers the shift from the vegetative to the reproductive growth stages in soybean (Ray et al., 2008). The length of darkness, and to a lesser extent, temperature, influences this change. In the Midwestern United States, soybean is typically planted from mid-April through mid-July. Early plantings may be exposed to late spring frosts and low soil temperatures that result in low germination and emergence, but may allow producers to harvest earlier and with increased seed yields. Later plantings may be exposed to early frosts in the fall, and could have the reproductive stages taking place during higher temperatures, resulting in more flower abortions and ultimately lower seed yields. However, this may also have the seed-filling period taking place during cooler temperatures, which can lead to a more advantageous seed composition profile and higher seed quality (Keim et al., 1999).

*Effects on yield:* Planting date has been shown to be a significant factor affecting soybean seed yield (Zhang et al., 2010); (Hu and Wiatrak, 2012). Generally, the effect of planting date on soybean seed yield is primarily dependent on both genetic and environmental conditions (Egli and Cornelius, 2009). Earlier planted soybeans typically show increased seed yields due to the extended duration of vegetative and reproductive growth stages (Chen and Wiatrak, 2010).

Additional research has also shown that soybean seed yield is correlated with the duration of certain physiological processes, including the length of flowering and pod set (Egli and Bruening, 2000) and seed filling (Andrade, 1995). Heatherly (2005) reported that a later planting date shortened the duration of both the vegetative and reproductive growth stages of maturity group (MG) IV through VI soybean cultivars. It was also shown that the most prominent difference between cultivars of different maturity groups was the length of the vegetative growth stage, rather than reproductive growth stages.

In order to maximize seed yield potential, it is important to establish an optimum planting date for a particular region and for different maturity groups. Numerous studies have been conducted for different regions of the United States in order to establish such guidelines. Egli and Cornelius (2009) evaluated experiments from three different regions of the United States (Midwest, Upper South and Deep South), and reported that soybean seed yield declined significantly when planted after the established optimum date, which varied from early May to early June. Overall, it is the combined effect of photoperiod, temperature and precipitation with late planting dates that primarily contribute to reduced duration of the vegetative and reproductive growth stages that ultimately lead to the significant reduction in seed yield of soybean.

*Effects on composition:* The environment in which a crop is grown can be manipulated by shifting the planting date. This can alter the range of temperatures, soil moisture levels, and day lengths that a crop is exposed to.

The biochemical processes involved in the synthesis and remobilization of seed composition components may be influenced by temperature, and therefore shifting the planting date may alter the composition of soybean seed. Additionally, changes in soil moisture levels may also affect composition by causing plants to perform osmotic adjustments that result in chemical element translocation (Samarah et al., 2004). Day length may alter composition by affecting the timing of transition from the vegetative to reproductive growth stages and ultimately the amount of dry matter during each of the two stages. This affects the amount of carbon assimilated by the plant and how it is partitioned among the plant (Cure et al., 1982) and the overall length of the seed-filling period (Thomas and Raper, 1976). Previous research has shown that both organic and inorganic seed components are significantly affected by temperature during the reproductive growth stages in soybean (Gibson and Mullen, 2001); (Ren et al., 2009); (Bellaloui et al., 2010); (Bellaloui, 2011); (Bellaloui et al., 2015).

Based on a review of the literature on this topic, the general consensus is that for genotypes of the same maturity group, planting soybeans later leads to the seed-filling period taking place in cooler temperatures than early planted soybeans, which can significantly affect seed composition. After reviewing multiple studies surrounding the effects of planting dates on compositional traits, many inconsistencies were observed.

Ray et al. (2008) evaluated agronomic traits and seed composition on eight soybean lines with modified fatty acid profiles. Early plantings in this study showed

significantly higher protein and total oil content than the late planting date, with significantly higher linolenic acid levels observed in the late planting date. Bellaloui (2011) conducted a study observing the influence of planting date on soybean seed composition under irrigated and non-irrigated conditions. Early plantings showed significantly higher total oil and oleic acid, with lower protein, linoleic and linolenic acids. Late plantings showed significantly higher sucrose and raffinose content, with lower stachyose content. Jaureguy et al. (2013) evaluated planting date and delayed harvest effects on seed composition using eight soybean lines with modified seed composition. Early plantings showed significantly higher protein and oleic acid concentrations, with a decrease in linolenic acid. Late plantings showed a significant increase in sucrose content. Bellaloui et al. (2015) evaluated the effects of planting date and other agricultural practices on soybean seed composition. Early plantings showed significant increases in total oil, oleic acid and sucrose. Late plantings showed significant increases in protein and linolenic acid, with lower oleic acid and total oil concentrations.

The results from this literature review show that the effects of planting date on soybean seed composition are inconsistent and inconclusive. Additionally, soybean seed sugar concentration has probably received the least amount of attention in planting date studies (Jaureguy et al., 2013), thus further research is needed to reconcile the controversial results from the existing literature.

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## **Chapter II**

# **Planting Date Effects on Agronomic Traits and Seed Composition of Soybeans with Modified Carbohydrate Profiles**

## **Abstract**

Information on management practices that can improve crop value can be useful for producers aiming to maximize farm profits. Differing planting dates alter the environment in which a crop is grown, and have previously been shown to significantly affect seed yield, composition, and quality. Providing soybean meal high in sucrose and low in the raffinose family of oligosaccharides (RFOs) is necessary for improving digestibility and feed efficiency in monogastric animals, and for increasing profitability for farmers by providing soybean varieties with value-added traits. In this study, the effects of planting date on soybean [*Glycine max* (L.) Merr.] seed composition and several agronomic traits were investigated using ten specialty genotypes with modified carbohydrate profiles due to known gene mutations, and with ten check varieties. The experiment was arranged in a split-plot design, with planting date as the main plot, and genotype as the subplot, performed in 2015 and 2016 at three locations each year in Missouri, each with three planting dates. Planting dates were chosen to simulate early, full season, and double-crop conditions. The environment had a significant effect on all traits measured, except protein. Planting date had a significant effect on all agronomic traits except lodging and seed weight, and all seed composition traits measured except RFOs. RFO concentrations were shown to be more stable when compared to sucrose concentrations. Early plantings showed increased yield and oil and late plantings showed increased sucrose and higher seed quality. Higher temperatures were shown to be associated with higher oil and RFO concentrations, while cooler temperatures were associated with higher sucrose concentrations. This research shows that a late planting or

double-cropping production system will provide the most optimum concentrations of high sucrose, low RFO soybeans compared to earlier planting dates.

## Introduction

Soybean [*Glycine max* (L). Merrill] is the most important oilseed crop in the world (Wilcox, 2004), and one of the leading economic crops grown in the United States. Soybean seeds consist of 40% protein, 20% oil, 35% carbohydrates, and 5% ash, on a dry weight basis (Hymowitz and Collins, 1974). Soybean meal is an important component of livestock feed, particularly for monogastric animals, due to its high protein content (~47%) and for being a great source of dietary fiber. In 2015, over 30 million metric tons of soybean meal were used for livestock feed, with poultry and swine using 16.3 (54%) and 6.9 (23%) million metric tons, respectively (ASA, 2016).

The three major soluble carbohydrates in soybean include sucrose, raffinose, and stachyose (Karr-Lilienthal et al., 2005); (Liu, 1997). The concentration of the soluble carbohydrates can play a role in determining the usefulness of soybean meal. Of the three major components, only sucrose is nutritionally beneficial and easily digested to be used as metabolizable energy for monogastric animals. Alternatively, the raffinose family of oligosaccharides (RFOs), primarily comprised of raffinose and stachyose, are considered as anti-nutritional factors in soybean meal because they are unable to be digested by monogastric animals due to the lack of the enzyme  $\alpha$ -galactosidase in the stomach and small intestine (Sebastian, 2000). The poor digestion of RFOs can lead to a reduction in metabolizable energy, ultimately resulting in poor weight gain and substantial loss in market value in livestock production operations (Rackis, 1974); (Zuo et al., 1996).

Deak et al. (2006) confirmed that genetic control offers a solution to removing unwanted RFOs in soybean. A soybean plant introduction, PI200508, was shown to have reduced levels of RFOs and increased sucrose, which was found to be due to reduced

raffinose synthase activity controlled by the mutant allele *stc1* (Sebastian, 2000). Soybean raffinose synthase activity has also been studied by Hitz et al. (2002) and Dierking and Bilyeu (2008), and these studies have shown that the soybean genome contains at least two expressed genes similar to other characterized raffinose synthases in other plants, raffinose synthase 2 (RS2) and raffinose synthase 3 (RS3), respectively. The novel allele of RS2 from PI200508 has been shown to completely associate with the low RFO phenotype. RS3 is also thought to contribute to the low RFO phenotype, but to a lesser degree when compared to the RS2 allele (Dierking and Bilyeu, 2008). The soybean line LR33 was shown to have reduced levels of RFOs, as well as an increase in sucrose, due to a novel allele of the myo-inositol phosphate-1 synthase gene (*MIPS1*), which plays an important role in the biosynthesis of raffinose and stachyose (Hitz et al., 2002) (Kerr et al., 2003).

Planting date is a management practice that can affect soybean growth and development, seed yield, and seed composition in soybean. The environment in which a crop is grown can be manipulated by changing the planting date, and this can change the range of temperatures, soil moisture levels, and day lengths that a crop is exposed to. Previous research has shown that for genotypes of the same maturity group, the later that soybeans are planted, the more likely the seed-filling period will take place in cooler temperatures than earlier planted soybeans, which can significantly affect seed composition (Ray et al., 2008); Bellaloui, 2011); (Jaureguy et al., 2013); (Bilyeu and Wiebold, 2016), and yield (Kandel et al., 2016); (Marburger et al., 2016); (Salmeron et al., 2016). Previous research has shown that delaying soybean planting led to significant increases in sucrose concentrations (Bellaloui, 2011); (Jaureguy et al., 2013). Hagely et

al. (2013) evaluated soybean lines with different combinations of alleles of the RS2 gene in one location. This study indicated variability between carbohydrate profiles of different genotypes, but showed that each genotypic class had a distinct carbohydrate profile. Another study, conducted by Bilyeu and Wiebold (2016), evaluated soybean lines with different combinations of mutant RS2 and RS3 alleles at different planting dates. This study showed significant increases in sucrose concentrations with later planting dates, and suggested that higher sucrose concentrations are produced with cooler temperatures during the seed-fill period. The objectives of this study are to expand upon previous research investigating the environmental stability of soybean lines with different sources of genetic variation for carbohydrate profiles. These include combinations of mutant RS2 and RS3 alleles, and a mutant MIPS1 allele across maturity groups III and IV at three locations in Missouri during 2015 and 2016.

## **Materials and Methods**

### **Field Experiment**

Eighteen soybean genotypes were grown in 2015 (Table 1); eight specialty lines bred for an improved carbohydrate profile, and ten check cultivars. Twenty genotypes were grown in 2016 (Table 2); ten specialty lines bred for an improved carbohydrate profile, and ten check cultivars. Genotypes were divided by maturity group (III and IV). The experiment was arranged in a split-plot design, with planting date as the main plot, and genotype as the subplot. This study was planted at three locations in Missouri during 2015 and 2016: the Bradford Research and Extension Center (REC) near Columbia (N38°53'36.80", W92°12'53.21), the Greenley Memorial REC near Novelty (N40°01'18.6", W92°11'25.3") and the Lee Farm REC near Portageville (N36°23'43.7",

W89°36'56.5"). Soil types are as follows: Bradford REC – Mexico silt loam (fine, smectitic, mesic, Aeric Vertic Epiaqualfs); Greenley Memorial REC – Putnam silt loam (fine, smectitic, mesic Vertic Albaqualfs); Lee Farm REC – Tiptonville silt loam (fine-silty, mixed, superactive, thermic Oxyaquic Argiudolls). Soybeans were rotated with corn (*Zea mays* L.) in 2015 and 2016 at all locations. Plots were planted with an ALMACO cone planter (ALMACO Inc. Nevada, IA) with four rows spaced at 0.76m. Plot dimensions were 4.88m by 2.29m. Planting dates were early May (early), late May/early June (mid), and late June/early July (late). Planting dates in 2015 were May 6, June 10, and June 24 for Columbia; June 5 and July 14 for Novelty; May 13, May 29, and June 16 for Portageville. Planting dates in 2016 were May 5, May 25, and June 15 for Columbia; May 24, June 15, and July 19 for Novelty; May 16, June 1, and June 8 for Portageville. In 2015, the early Novelty planting date was lost due to excessive rainfall and pest pressure.

### **Agronomic Traits**

Agronomic traits that were recorded included: flower color, pubescence color, maturity, height, lodging, yield, moisture, and 100-seed weight. Flower and pubescence color were recorded based on visual observations when >90% of all plants within a plot were flowering. Maturity at R8 (Fehr et al., 1971) was taken when at least 95% of all pods were fully mature within each plot and scored based on the number of days after September 1st. Height was recorded at maturity and measured in cm. from ground level to the average height of the plants within the plot. Lodging was recorded at maturity and scored on a 1-5 scale (1= all plants fully erect; 5= all plants on the ground). Yield and moisture content were recorded simultaneously during harvest of the middle two rows by an ALMACO SPC-40 plot combine (ALMACO, Inc. Nevada, IA). 100-seed weight was

measured in grams by taking the weight of 100 seeds as measured by a Mettler-Toledo precision balance (Mettler-Toledo International, Inc. Columbus, OH).

### **Seed Composition Analysis**

*Protein and oil analysis:* 100 g samples of seed from each plot was used for protein and oil analysis using a FOSS (Eden Prairie, MN) XDS Rapid Content Analyzer near infrared reflectance (NIR) spectroscopy instrument at the Bay Farm Research Facility in Columbia, MO. The operation method included each sample being scanned thirty-two times, and an average of all scans presented. Protein and oil concentrations were presented as a percentage, and subsequently converted to milligrams per gram ( $\text{mg g}^{-1}$ ). Moisture content of each sample was adjusted to 13%.

*Carbohydrate analysis:* Soluble carbohydrates sucrose, raffinose, and stachyose were quantified by high performance liquid chromatography (HPLC) equipped with an evaporative light scattering detection (ELSD) system, as described by Valliyodan et al. (2015), with slight modifications. Around 10 seeds from each field plot were randomly selected and ground, and the ground powder was lyophilized for 48 hours in a Labconco Freeze Dry System (Labconco, USA). Soluble carbohydrates of the seed were extracted by an acetonitrile extraction method. Briefly, about 0.09 g (0.0895-0.0905 g) of the dried sample was weighed and mixed with 900  $\mu\text{L}$  HPLC grade water in 2 mL centrifuge vials. The vials were incubated at 55°C for 30 minutes at 200 rpm agitation, followed by 20 seconds of high speed vortex. 900  $\mu\text{L}$  of 95% HPLC grade acetonitrile was then blended in, with an additional 20 seconds of high speed vortex. Samples were then centrifuged at 10000 g for 10 minutes. 800  $\mu\text{L}$  of the supernatant was then filtered through a 0.45  $\mu\text{m}$

filter. 100  $\mu\text{L}$  of the sample solution was finally mixed with 400  $\mu\text{L}$  of 65% HPLC grade acetonitrile in a 2 mL glass HPLC vial. Sugar standards for sucrose, raffinose, and stachyose from Sigma-Aldrich (St. Louis, MO) were prepared in water with concentrations of 50, 100, 300, 500, and 1000  $\mu\text{g}/\text{mL}$ . The HPLC system used for this experiment was an Agilent 1200 series (Agilent, USA). The separation of the soluble carbohydrates was performed by a Prevail Carbohydrate ES column (5  $\mu\text{m}$  250 mm x 4.6 mm), coupled to a guard column (7.5 x 4.6 mm), from Grace Davison Discovery Sciences (Deerfield, IL). Two mobile phases were prepared: mobile phase A was 100% HPLC grade water, and mobile phase B was an acetonitrile: acetone mixture of 75:25 (v/v), with a flow rate of 1.2 mL/min. Column temperature was maintained at 35°C, and the detector temperature was isothermal at 55°C. Nebulizer pressure was maintained at 3.4 bar, with ultrapurity-grade nitrogen used as the nebulizer gas. Sample injections were made at a volume of 5  $\mu\text{L}$ . Sample run time was a total of 18 minutes, with the first 14 minutes for sample separation followed by a 4 minute reconditioning step. Sucrose, raffinose, and stachyose were predicted based on standard curves generated for each carbohydrate. Concentrations of sucrose, raffinose, and stachyose are presented in milligrams of sugar per gram of seed ( $\text{mg g}^{-1}$ ). A chromatograph example of each genotypic type's carbohydrate profile is shown in Figure 1. A chromatograph example of seed carbohydrate response to planting date is shown in Figure 2, with signals from each planting date overlaid onto one plot.

## **Statistical Analysis**

Based on the combination of alleles, genotypes were grouped into four different categories, referred to as ‘types’: genotypes containing a mutant RS2 allele (rs2), genotypes containing a combination of mutant RS2 and RS3 alleles (rs2/rs3), genotypes containing the mutant MIPS1 allele (mips1), and genotypes containing wild type alleles (checks). The combination of years and locations were considered as five different environments included in the statistical analysis. This experiment was analyzed as a split-split plot, with environment as the whole plot, planting date as the split-plot, and genotype as the split-split plot. All factors were considered fixed in the analysis, except for plot within location (plot (location)), and plot within location at each planting date (plot (location planting date)). Analysis of variance (ANOVA) was conducted over all environments using PROC GLIMMIX procedure in SAS 9.3 (SAS Institute, 2011). Least squares means were estimated using the lsmeans option, and significant differences between the least squares means were computed by using the lines option. Pearson correlation coefficients were calculated using the PROC CORR procedure in SAS 9.3 (SAS Institute, 2011).

## **Results and Discussion**

### **Planting Date Effects on Agronomic Traits**

Analysis of variance (ANOVA) was conducted to identify environment, planting date, and type effects on all agronomic traits measured (height, lodging, maturity, seed quality, seed weight, and yield). The overall ANOVA showed that the environment (Env) effect was significant on all agronomic traits (Table 3). The planting date (PD) effect was

significant on all agronomic traits except lodging. The Env x PD interactions were significant on all agronomic traits except seed quality and seed weight. The type (T) effect was significant for all agronomic traits except lodging. The Env x T effect was significant for all agronomic traits, and the PD x T effect was significant for all agronomic traits except lodging and seed weight. The Env x PD x T effect was only significant on height. There were no other significant effects (Table 3).

Overall, late plantings led to significantly higher seed quality compared to the early and mid-plantings, and late plantings had significantly lower height and yield (Table 4). These results were consistent with previous research on soybean yields at different planting dates (Egli and Cornelius, 2009); (Chen and Wiatrak, 2010); (Hu and Wiatrak, 2012; Kandel et al., 2016; Marburger et al., 2016; Salmerón et al., 2016). Within genotypic types, these trends were the same, with the exception of the mips1 group, which had significantly higher height at late plantings compared to the early and mid-plantings. The mips1 group showed no significant differences in yield across the different planting dates (Table 4). This is most likely due to the late maturity of the two genotypes in the mips1 group for the environments in which this study was conducted. The checks group had significantly higher yields overall compared to the other groups, with the rs2/rs3 group having significantly higher yields than the mips1 and rs2 groups (Figure 1). The rs2 group had significantly lower height as compared to the other groups, which were not significantly different. The mips1 group had significantly worse seed quality scores as compared to the other groups, which were not significantly different. The rs2 group showed significantly higher seed weight as compared to the other groups, with the

rs2/rs3 and mips1 groups both having significantly higher seed weight than the checks group (Figure 1).

Overall, these trends remained the same across locations, with the exception of the Portageville locations. At these locations, height showed significant increases with later plantings as compared to the earliest planting date. In the 2015 Portageville location, yield showed no significant differences among planting dates, with the exception of the rs2/rs3 group, which showed significant increases in yield at the latest planting date as compared to the early planting dates (Tables 6, 9). The deviations from the overall trends at this location are most likely due to the extended growing season at the Portageville research station, which is the southernmost location in this study.

These results provide valuable information on the agronomic performance of the specialty genotypes represented in this study, as well as adding to the existing literature on the effects of planting date on the agronomic performance of commodity-type cultivars.

### **Planting Date Effects on Seed Composition**

Analysis of variance was conducted to identify environment, planting date, and type effects on all seed composition traits measured (protein, oil, sucrose, and RFOs). The overall ANOVA showed that the environment (Env) effect was significant on all seed composition traits measured with the exception of protein (Table 3). The planting date (PD) effect was significant on protein, oil, and sucrose; but not significant on raffinose, stachyose, or combined RFOs. The Env x PD effect was only significant on protein and oil. The type (T) effect was significant on all seed composition traits. The

significant variance between genotypic groups was expected, due to this study containing both commodity type cultivars, and specialty lines bred for an improved carbohydrate profile. The Env x T effect was significant on all seed composition traits except protein. The PD x T effect was significant on raffinose and stachyose only (Table 3). There were no other significant effects.

Overall, planting date had a significant effect on protein concentrations, but there were no clear trends observed. Increased protein content is generally associated with higher temperatures (Burton, 1987). However, protein generally increased with later plantings as compared to earlier plantings in the rs2/rs3 and checks group, while the opposite trend was observed for the mips1 and rs2 groups (Tables 4–9). Oil was significantly higher in the early plantings as compared to later plantings (Table 4). These results further reinforce the negative correlation observed between protein and oil content in previous research (Geater and Fehr, 2000); (Wilcox, 2004); (Bellaloui, 2011); (Jaureguy et al., 2013); (Bellaloui et al., 2015). Sucrose was significantly higher in late plantings as compared to earlier plantings. Concentrations of RFOs were not significantly different at any of the three planting dates. These results are consistent with previous research on planting date effects on soybean carbohydrate profiles (Bellaloui, 2011); (Jaureguy et al., 2013); (Bilyeu and Wiebold, 2016). Within genotypic types, these trends generally stayed the same, with the exception of protein. Protein was significantly higher at later plantings as compared to early plantings in the rs2/rs3 and checks group, with protein being significantly higher in the early planting in the mips1 group, and no significant differences between the early and late plantings in the rs2 group. For all seed composition traits, each genotypic type was significantly different from the others (Figure

1). For protein, the rs2/rs3 group had the highest concentrations, followed by: rs2, mips1, and checks, respectively. These trends were as expected, due to the fact that the genotypes in the rs2/rs3 group were also bred for high protein content. For oil, the checks group had the highest oil concentrations, followed by: rs2, mips1, and rs2/rs3, respectively. For sucrose, the mips1 group had the highest concentrations, followed by: rs2/rs3, rs2, and checks, respectively. For RFOs, the checks had the highest concentrations, followed by: rs2, rs2/rs3, and mips1, respectively (Figure 1).

These results indicate that the accumulation of carbohydrate components is genotype dependent. Planting date also showed a significant effect on sucrose concentrations, with the late planting having significantly higher concentrations than both the early and mid-planting. These trends were true for all genotypic types, thus showing that later planting dates will favor both commodity-type and specialty genotypes for the production of high sucrose soybean meal to meet specialty market demands. The mips1 group exhibited the most advantageous carbohydrate profile to meet specialty market demands, by having the highest sucrose concentrations overall, and also having the lowest concentrations of RFOs (Figure 1). The rs2/rs3 group had the second highest concentration of sucrose and the second lowest concentrations of RFOs, both significantly better than the group with only the rs2 mutant allele, and all exhibiting a significantly improved carbohydrate profile as compared to the check group.

These results provide useful information on the environmental stability of the high sucrose/low RFO trait in different genetic backgrounds, as well as providing further evidence of how seed composition of commodity-type cultivars are affected by different

environments. This research can also prove to be useful to soybean producers that are growing soybeans for specialty markets that must meet certain quality standards.

### **Weather Data Associations with Seed Composition Components**

Based on previous research, temperature during the pod-filling period is the primary environmental factor affecting seed composition (Kane et al., 1997; Piper and Boote, 1999; Wolf et al., 1982). During the two years of this study, 2015 had lower temperatures overall, and during August – October, during which the pod-filling period took place across the varying planting dates (Table 2). Location years were considered as separate locations for this study. The Portageville locations showed the highest temperatures overall, followed by the Columbia and Novelty locations, respectively. These temperature trends were consistent with many observed trends for the seed composition components measured. Overall, sucrose concentrations were higher at the Columbia and Novelty locations (Tables 5, 7, 8) (Figures 4, 6, 7) as compared to the Portageville locations (Tables 6, 9) (Figures 5, 8). This reinforces previous research showing cooler temperatures during pod-fill associating with sucrose accumulation (Bellaloui, 2011); (Jaureguy et al., 2013); (Bilyeu and Wiebold, 2016). Conversely, RFO concentrations were highest at the Portageville locations, where temperatures were highest. Oil concentrations were highest at the Portageville and Columbia locations (Figures 4, 5, 6, 8), where temperatures were higher as compared to the Novelty location (Figure 7). This is consistent with previous research, showing that warmer temperatures associated with early plantings should favor high oil concentrations (Dardanelli et al., 2006; Kane et al., 1997; Ray et al., 2008; Robinson et al., 2009). Protein showed no

significant environment effect, and there were no clear trends observed within each location.

### **Correlations among Traits**

Pearson correlation coefficients were calculated at each planting date between all agronomic traits and seed composition constituents evaluated in this study. For the agronomic traits evaluated, height showed a significant positive correlation with lodging at all three planting dates (Tables 10, 11, 12). Seed quality showed significant negative correlations with height in the first and second planting dates, and a significant negative correlation with lodging in the first and second planting dates. There were significant negative correlations between seed weight and maturity at the second and third planting dates. The only significant correlations with yield were with RFO concentrations, which showed a strong positive correlation at all three planting dates (Tables 10, 11, 12).

There were many significant correlations found between seed composition constituents at all planting dates. Protein and oil showed a significant negative correlation ( $p < 0.01$ ) at all planting dates (Tables 9, 10, 11), confirming previous research (Geater and Fehr, 2000); (Wilson, 2004); (Bellaloui, 2011); (Jaureguy et al., 2013). Oil also showed a significant negative correlation with sucrose concentration across all plantings. RFO concentrations showed a significant positive correlation with oil, and significant negative correlations with protein and sucrose concentrations (Tables 10, 11, 12).

Results from this study indicate that producing high sucrose, low RFO soybean meal is most easily attained through delayed plantings in the spring and planting varieties that contain a combination of mutant RS2/RS3 alleles, or a mutant MIPS1 allele.

Delaying planting can also significantly increase sucrose content in commodity-type soybeans. These results can provide useful information to producers aiming to maximize sucrose accumulation while lowering concentrations of RFOs in order to gain premiums from meeting specialty market demands. However, the specialty genotypes evaluated in this study yielded significantly less than the commodity-type checks, and thus, more research is needed to determine whether it is economically feasible at this time to utilize these specialty genotypes in a double-crop production system in order to improve profits, or if it will be necessary to incorporate these traits into higher yielding backgrounds. The next chapter of this thesis will begin to evaluate the economic feasibility of utilizing these specialty genotypes.

## Tables

**Table 1.** Entry list of soybean genotypes with listed allelic mutation used in the planting date study.

<b>Genotype</b>	<b>Type†</b>	<b>MG</b>	<b>Developing Institution</b>
XD3553	rs2/rs3	3	Schillinger Genetics, Inc.
KB12-31#1027	rs2/rs3	3	U.S. Dept. of Agriculture
e3011	rs2	3	Schillinger Genetics, Inc.
IA3051HS	rs2	3	Iowa St. Univ.
IA2104HS	rs2	3	Iowa St. Univ.
IA3023	Check	3	Iowa St. Univ.
IA3024	Check	3	Iowa St. Univ.
LD07-3395bf	Check	3	Univ. of Illinois
AG3231	Check	3	Monsanto Company
NK S39-U2	Check	3	Syngenta AG
R07-2000	mips1	4	Univ. of Arkansas
R07-2001	mips1	4	Univ. of Arkansas
KB13-7#1102	rs2/rs3	4	U.S. Dept. of Agriculture
CR13-399	rs2	4	Univ. of Missouri
CR14-443	rs2	4	Univ. of Missouri
444D212P	Check	4	Schillinger Genetics, Inc.
Ellis	Check	4	UniSouth Genetics, Inc.
AG4232	Check	4	Monsanto Company
AG4632	Check	4	Monsanto Company
94Y23	Check	4	DuPont Pioneer

† Type = type of genotype based on allelic mutation (MIPS1, rs2, rs2/rs3, checks).

**Table 2.** Planting dates and monthly average of daily maximum air temperature (T max in °C), daily minimum air temperature (T min in °C), and total monthly rainfall (mm) for each growing season and environment in which the genotypes grown for this study were evaluated.

		2015			2016		
		Columbia	Novelty	Portageville	Columbia	Novelty	Portageville
<b>May</b>	T max	23.3	22.7	25.9	22.8	21.9	24.4
	T min	13.0	12.1	15.8	11.0	10.9	14.3
	Rainfall	140	119	126	81	108	94
<b>June</b>	T max	27.9	27.5	31.6	30.7	30.4	31.4
	T min	18.7	17.5	21.4	18.8	18.0	20.8
	Rainfall	130	322	91	29	38	50
<b>July</b>	T max	29.7	29.4	32.6	30.0	28.9	32.0
	T min	20.1	19.0	23.2	19.9	18.9	22.4
	Rainfall	204	257	70	274	116	90
<b>August</b>	T max	28.5	27.9	30.5	28.9	28.3	30.4
	T min	17.4	16.9	19.0	19.2	18.8	21.8
	Rainfall	106	106	50	149	206	137
<b>September</b>	T max	27.7	27.5	28.5	27.3	26.6	29.6
	T min	15.6	14.9	16.0	15.5	15.1	16.9
	Rainfall	21	35	19	143	45	29
<b>October</b>	T max	20.4	19.3	22.1	22.3	21.2	25.7
	T min	7.8	6.9	9.7	10.2	9.8	12.2
	Rainfall	25	52	73	25	52	30
<b>Planting Dates</b>	Early	May 6	—	May 13	May 5	May 24	May 16
	Mid	June 10	June 5	May 29	May 25	June 15	June 1
	Late	June 24	July 14	June 16	June 15	July 19	June 8

**Table 3.** Overall Analysis of variance (ANOVA) for agronomic and seed composition traits for twenty genotypes grown in 2015 and 2016; ten specialty lines bred for an improved carbohydrate profile, and ten check cultivars.

<b>Source of Effects</b>	<b>Ht§</b>	<b>Lod</b>	<b>Mat</b>	<b>SDQT</b>	<b>SDWT</b>	<b>Pro</b>	<b>Oil</b>	<b>Suc</b>	<b>Raff</b>	<b>Stac</b>	<b>RFOs</b>	<b>Yield</b>
Environment (Env)	***	***	***	***	***	NS	***	***	***	***	***	***
Planting Date (PD)	***	NS	***	***	NS	**	***	**	NS	NS	NS	***
Env x PD	***	***	**	NS	NS	**	**	NS	NS	NS	NS	***
Type (T)†	***	NS	***	***	***	***	***	***	***	***	***	***
Env x T	***	***	***	***	***	NS	***	***	***	***	***	***
PD x T	***	NS	***	**	NS	NS	NS	NS	*	*	NS	***
Env x PD x T	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

† Type = type of genotype based on allelic mutation (MIPS1, rs2, rs2/rs3, checks).

§ Ht = height (cm); Lod = lodging score (1 = all plants erect, 5 = all plants down); Mat = maturity (days after September 1);

SDQT = seed quality score (1 = best, 5 = worst); SDWT = 100-seed weight (g); Pro = protein concentration (mg g<sup>-1</sup>); Oil = oil concentration (mg g<sup>-1</sup>); Suc = sucrose concentration (mg g<sup>-1</sup>); Raff = raffinose concentration (mg g<sup>-1</sup>); Stac = stachyose concentration (mg g<sup>-1</sup>); RFOs = Raffinose Family of Oligosaccharides (raffinose + stachyose) (mg g<sup>-1</sup>); Yield = seed yield (kg ha<sup>-1</sup>).

NS not significant at the 0.05 probability level.

\* significant at the 0.05 probability level.

\*\* significant at the 0.01 probability level.

\*\*\* significant at the 0.001 probability level.

**Table 4.** Agronomic traits and seed composition of twenty genotypes as influenced by planting date (early, mid, late) averaged across environments, years, and maturity group. Included are: ten specialty lines bred for an improved carbohydrate profile, and ten check cultivars.

Type†	Ht‡	Lod	SDQT	SDWT	Pro	Oil	Suc	RFOs	Yield
<b>Check</b>									
Early	91.8	2.4	2.2	14.1	341.7	189.9	47.0	52.2	4279.8
Mid	93	2.4	2.0	14.1	339.6	190.8	49.5	53.7	4132.3
Late	83.9	2.4	2.1	14.0	346.4	185.5	50.9	54.3	3322.5
<b><u>mips1</u></b>									
<u>Early</u>									
R07-2000	77.7	2.1	2.4	15.4	374.5	170.8	95.5	8.9	2819.8
R07-2001	86.7	2.3	2.7	14.1	356.0	175.0	84.4	13.9	2905.7
Mean	83.8	2.3	2.5	14.8	365.2	172.2	90.5	11.7	2884.4
<u>Mid</u>									
R07-2000	88.2	1.9	2.1	14.6	364.2	169.2	99.1	9.6	3126.9
R07-2001	91.7	2.5	2.2	14.1	353.5	175.0	83.5	17.8	3034.2
Mean	89.8	2.3	2.2	14.4	358.9	172.1	91.7	13.8	2916.4
<u>Late</u>									
R07-2000	91.6	2.3	2.5	14.7	364.7	166.4	105.0	9.2	2996.1
R07-2001	96.3	2.4	2.5	13.8	359.2	164.8	88.4	15.1	2988.4
Mean	93.6	2.4	2.5	14.2	361.9	165.3	95.7	11.9	2835.2
<b><u>rs2</u></b>									
<u>Early</u>									
e3011	94.6	2.2	2.4	14.5	417.2	162.1	57.7	21.7	4047.1
IA2104HS	72.2	2.1	2.7	19.6	372.3	184.9	67.0	24.9	3050.6
IA3051HS	81.7	2.2	2.7	18.7	384.3	175.0	66.8	31.4	3194.0
CR13-399	99.3	3.0	2.0	15.0	359.6	195.7	48.3	25.8	2763.8
CR14-443	111.7	3.3	2.1	14.2	363.9	197.7	46.6	24.3	2458.3
Mean	88.3	2.4	2.5	16.5	381.7	182.3	57.1	25.5	3058.0
<u>Mid</u>									
e3011	94.2	2.4	2.0	14.5	409.2	164.7	60.2	20.2	3846.3
IA2104HS	72.0	1.9	2.1	18.7	364.7	187.9	66.6	24.4	2953.0
IA3051HS	87.5	2.3	2.1	18.2	375.0	180.0	70.6	32.1	3419.3
CR13-399	91.7	2.2	2.0	15.1	357.3	195.8	50.8	25.9	2270.9
CR14-443	109.7	2.8	1.7	15.1	354.0	199.8	51.9	24.1	2538.4
Mean	88.5	2.3	2.0	16.3	372.2	185.5	59.8	25.3	3014.2
<u>Late</u>									
e3011	83.2	2.4	1.8	15.3	419.2	160.5	64.4	21.5	3149.0

IA2104HS	73.2	2.1	2.0	18.2	371.5	182.6	74.0	24.6	2571.0
IA3051HS	75.3	2.6	2.0	18.5	380.7	176.5	63.8	26.6	2927.9
CR13-399	90.5	2.4	2.0	14.5	363.4	188.7	54.2	25.8	1738.9
CR14-443	96.5	2.7	1.8	13.9	360.3	191.9	55.3	24.5	2264.4
Mean	80.0	2.3	2.0	16.1	379.4	180.0	62.4	24.2	2472.8
<b><u>rs2/rs3</u></b>									
<b><u>Early</u></b>									
XD3553	90.7	1.9	2.2	15.2	391.6	162.5	64.0	23.0	4472.5
KB12-31#1027	108.5	3.2	2.0	13.2	389.7	177.4	74.2	20.5	3225.4
KB13-7#1102	121.6	3.2	1.8	13.3	369.8	191.2	65.9	20.0	3222.0
Mean									
<b><u>Mid</u></b>									
XD3553	97.1	2.3	1.9	14.7	389.1	168.0	67.1	21.5	3895.4
XD3553	90.4	1.8	1.8	15.3	390.1	164.1	69.0	23.9	4370.3
KB12-31#1027	114.6	3.2	1.6	14.1	391.8	175.7	67.6	17.3	3308.2
KB13-7#1102	116.9	2.9	1.8	14.0	361.0	192.9	64.4	21.3	2833.8
Mean	94.9	2.3	2.3	14.4	391.1	167.3	65.7	22.4	4012.6
<b><u>Late</u></b>									
XD3553	80.6	2.3	2.0	15.2	399.1	161.7	71.6	26.4	3417.8
KB12-31#1027	96.6	3.2	1.5	14.4	395.7	172.6	68.6	18.0	2591.7
KB13-7#1102	103.3	3.0	1.8	13.0	371.9	185.3	66.5	19.4	2535.3
Mean	84.5	2.5	2.0	15.1	397.8	164.1	69.4	23.5	3238.1
<b>LSD§</b>	1.9	0.09	0.09	0.3	2.8	1.7	1.9	1.3	121.1

† Type = type of genotype based on allelic mutation.

‡ Ht = height (cm); Lod = lodging score (1 = all plants erect, 5 = all plants down);

SDQT = seed quality score (1 = best, 5 = worst); SDWT = 100-seed weight (g);

Pro = protein concentration ( $\text{mg g}^{-1}$ ); Oil = oil concentration ( $\text{mg g}^{-1}$ ); Suc = sucrose concentration ( $\text{mg g}^{-1}$ ); RFOs = Raffinose Family of Oligosaccharides (raffinose + stachyose) ( $\text{mg g}^{-1}$ ); Yield = seed yield ( $\text{kg ha}^{-1}$ ).

§ LSD = least significant difference at the 0.05 probability level.

**Table 5.** Agronomic traits and seed composition of eighteen genotypes as influenced by planting date (early, mid, late) at Columbia in 2015. Included are: nine specialty lines bred for an improved carbohydrate profile, and nine check cultivars.

2015 Columbia									
Type†	Ht‡	Lod	SDQT	SDWT	Pro	Oil	Suc	RFOs	Yield
<b>Check</b>									
Early	78.0	2.0	2.9	14.2	341.9	186.5	42.4	44.9	4551.9
Mid	70.7	1.6	2.7	13.0	334.9	188.6	42.6	47.0	3771.7
Late	67.4	1.6	2.4	13.0	347.9	180.7	44.8	48.0	2918.4
<b>mips1</b>									
<u>Early</u>									
R07-2000	77.3	2.0	4.0	14.0	392.3	152.0	74.3	9.3	1952.5
R07-2001	88.7	2.0	4.3	12.0	372.7	153.3	62.1	12.2	1544.5
Mean	83.0	2.0	4.2	13.0	382.5	152.7	68.5	10.8	1748.5
<u>Mid</u>									
R07-2000	63.7	1.5	2.7	13.1	350.7	165.3	90.8	13.1	2111.7
R07-2001	54.7	1.5	3.7	12.6	354.5	163.5	61.6	11.8	1681.3
Mean	59.2	1.5	3.2	12.9	352.2	164.6	76.3	12.4	1896.5
<u>Late</u>									
R07-2000	76.2	1.5	3.6	13.8	370.2	148.7	93.7	9.7	1970.9
R07-2001	85.4	1.5	4.3	10.6	371.1	134.4	57.8	12.2	2294.8
Mean	80.5	1.5	4.0	12.2	370.8	141.5	75.7	11.0	2135.2
<b>rs2</b>									
<u>Early</u>									
e3011	82.3	1.7	3.3	14.8	416.8	157.0	53.7	21.5	4252.4
IA2104HS	66.3	1.5	3.0	19.1	377.0	174.3	67.0	23.0	3001.6
IA3051HS	71.0	1.5	3.6	20.6	391.6	165.7	59.9	23.8	3391.6
CR13-390	52.3	1.5	3.7	17.3	389.7	182.3	51.6	19.1	1257.6
CR13-399	80.7	2.2	2.4	14.1	344.7	194.3	53.6	22.5	3238.1
Mean	70.5	1.7	3.2	17.2	384.0	174.7	57.2	22.0	3013.3
<u>Mid</u>									
e3011	73.7	1.7	2.0	13.7	406.0	161.8	52.1	17.4	3893.8
IA2104HS	61.0	1.5	2.6	16.4	354.2	181.2	49.4	19.0	2706.8
IA3051HS	69.7	1.5	2.7	17.2	363.2	179.2	56.5	22.4	3853.4
CR13-390	55.5	1.5	1.7	15.3	360.7	191.7	49.2	18.3	1526.6
CR13-399	60.3	1.5	3.0	14.3	353.3	188.0	51.4	22.0	—
Mean	64.6	1.5	2.4	15.4	367.5	180.4	51.6	19.8	2995.6
<u>Late</u>									
e3011	66.7	1.5	2.4	14.0	416.9	155.2	53.6	18.9	2717.2
IA2104HS	54.5	1.5	2.7	17.0	371.4	177.5	57.8	19.4	1688.0

IA3051HS	60.2	1.3	2.4	18.0	376.5	171.6	55.1	22.5	2817.0
CR13-390	47.4	1.5	3.0	14.3	374.7	182.3	53.4	16.3	1028.9
CR13-399	61.7	1.6	3.1	13.2	361.9	181.4	51.4	20.8	1536.2
Mean	57.4	1.5	2.7	15.3	380.2	173.6	54.4	19.6	2146.6
<b>rs2/rs3</b>									
<u>Early</u>									
XD3553	72.3	1.5	2.7	14.2	390.2	162.1	53.1	20.1	4270.4
<u>Mid</u>									
XD3553	71.7	1.5	2.3	14.7	380.8	163.3	62.5	22.0	4109.0
<u>Late</u>									
XD3553	63.7	1.5	3.0	14.1	396.5	159.4	61.3	21.7	2922.0
<b>LSD§</b>	3.4	0.12	0.3	0.7	6.7	4.0	3.8	2.3	300.0

† Type = type of genotype based on allelic mutation.

‡ Ht = height (cm); Lod = lodging score (1 = all plants erect, 5 = all plants down);

SDQT = seed quality score (1 = best, 5 = worst); SDWT = 100-seed weight (g);

Pro = protein concentration ( $\text{mg g}^{-1}$ ); Oil = oil concentration ( $\text{mg g}^{-1}$ ); Suc = sucrose concentration ( $\text{mg g}^{-1}$ ); RFOs = Raffinose Family of Oligosaccharides (raffinose + stachyose) ( $\text{mg g}^{-1}$ ); Yield = seed yield ( $\text{kg ha}^{-1}$ ).

§ LSD = least significant difference at the 0.05 probability level.

**Table 6.** Agronomic traits and seed composition of eighteen genotypes as influenced by planting date (early, mid, late) at Portageville in 2015. Included are: nine specialty lines bred for an improved carbohydrate profile, and nine check cultivars.

2015 Portageville									
Type†	Ht‡	Lod	SDQT	SDWT	Pro	Oil	Suc	RFOs	Yield
<b>Check</b>									
Early	86.4	2.3	2.7	13.7	343.4	190.1	44.0	54.9	4484.4
Mid	97.6	2.6	2.5	14.0	337.6	192.3	46.6	57.1	4542.8
Late	99.9	2.4	2.6	14.2	342.6	189.5	48.0	56.4	4434.8
<b>mips1</b>									
<u>Early</u>									
R07-2000	83.5	2.0	2.6	16.2	377.3	171.2	102.7	10.9	3942.5
R07-2001	87.2	2.3	2.7	15.4	360.5	177.2	89.5	13.0	3929.9
Mean	85.5	2.2	2.7	15.8	366.9	174.2	96.1	11.9	3852.1
<u>Mid</u>									
R07-2000	97.1	2.7	2.6	15.4	368.8	169.4	96.6	10.2	3909.8
R07-2001	99.5	3.0	2.4	14.4	357.4	176.9	81.4	22.9	3520.9
Mean	98.2	2.8	2.5	14.9	362.6	173.3	88.8	16.5	3710.8
<u>Late</u>									
R07-2000	110.7	3.0	2.9	15.1	359.2	172.6	105.7	8.7	3712.4
R07-2001	113.5	2.7	2.7	15.1	362.7	173.0	86.4	16.1	3816.6
Mean	111.8	2.8	2.8	15.1	359.2	172.9	95.0	12.4	3711.2
<b>rs2</b>									
<u>Early</u>									
e3011	90.3	2.7	3.0	13.9	425.8	158.8	48.9	25.4	4255.2
IA2104HS	65.9	2.0	4.0	19.2	375.8	186.7	56.7	24.8	3124.2
IA3051HS	66.2	2.0	3.3	18.2	393.1	176.1	52.0	29.5	3314.5
CR13-390	46.2	1.0	3.3	14.9	393.1	194.9	30.5	24.0	1332.7
CR13-399	89.9	3.0	2.0	14.2	358.3	199.6	45.2	31.2	4716.4
Mean	71.6	2.1	3.1	16.1	389.9	183.0	47.2	27.0	3263.9
<u>Mid</u>									
e3011	96.5	3.0	2.7	14.6	416.2	162.0	52.5	24.3	4088.6
IA2104HS	76.8	2.0	2.7	18.7	353.8	194.3	66.6	28.7	2976.9
IA3051HS	84.8	2.3	3.0	18.5	381.0	177.9	60.0	30.1	3109.4
CR13-390	72.2	2.0	2.0	14.9	348.5	209.3	48.0	23.8	1723.6
CR13-399	102.9	2.3	2.3	14.1	346.5	203.3	48.3	31.8	3559.4
Mean	86.7	2.3	2.5	16.2	368.7	189.4	54.7	27.8	3163.9
<u>Late</u>									
e3011	98.2	2.3	2.4	15.7	415.6	161.5	61.0	27.1	3929.2
IA2104HS	88.1	2.0	2.4	18.9	366.3	186.9	67.0	29.6	3610.7

IA3051HS	87.3	2.3	2.0	18.8	387.1	181.4	59.9	30.0	3995.1
CR13-390	79.3	2.0	2.0	15.6	352.6	201.4	51.8	22.8	1931.9
CR13-399	102.6	2.3	1.9	15.4	356.3	201.8	51.6	24.5	1917.6
Mean	90.4	2.2	2.2	16.9	375.0	187.1	58.4	26.8	3084.5
<b>rs2/rs3</b>									
<u>Early</u>									
XD3553	81.3	2.0	3.0	14.3	396.0	160.8	57.5	26.6	4249.8
<u>Mid</u>									
XD3553	92.3	2.0	2.7	14.7	382.6	167.6	61.5	26.7	4256.1
<u>Late</u>									
XD3553	94.6	2.0	2.4	16.5	400.2	161.6	62.6	29.3	4756.9
<b>LSD§</b>	4.0	0.2	0.2	0.6	7.0	4.2	3.2	2.4	293.2

† Type = type of genotype based on allelic mutation.

‡ Ht = height (cm); Lod = lodging score (1 = all plants erect, 5 = all plants down);

SDQT = seed quality score (1 = best, 5 = worst); SDWT = 100-seed weight (g);

Pro = protein concentration ( $\text{mg g}^{-1}$ ); Oil = oil concentration ( $\text{mg g}^{-1}$ ); Suc = sucrose concentration ( $\text{mg g}^{-1}$ ); RFOs = Raffinose Family of Oligosaccharides (raffinose + stachyose) ( $\text{mg g}^{-1}$ ); Yield = seed yield ( $\text{kg ha}^{-1}$ ).

§ LSD = least significant difference at the 0.05 probability level.

**Table 7.** Agronomic traits and seed composition of twenty genotypes as influenced by planting date (early, mid, late) at Columbia in 2016. Included are: ten specialty lines bred for an improved carbohydrate profile, and ten check cultivars.

2016 Columbia									
Type†	Ht‡	Lod	SDQT	SDWT	Pro	Oil	Suc	RFOs	Yield
<b>Check</b>									
Early	102.3	2.7	1.9	14.4	341.2	190.2	52.2	53.7	4742.8
Mid	108.3	2.9	1.5	14.4	343.6	189.0	55.1	54.3	4791.1
Late	92.9	3.7	1.7	14.9	342.2	188.9	58.1	55.1	3497.7
<b>mips1</b>									
<u>Early</u>									
R07-2000	77.7	2.7	1.7	17.9	367.7	173.5	110.8	8.7	3548.6
R07-2001	88.3	2.3	2.3	16.2	351.8	178.9	113.7	10.5	3947.6
Mean	83.0	2.5	2.0	17.1	359.7	176.2	111.7	9.4	3748.1
<u>Mid</u>									
R07-2000	96.0	2.1	1.5	17.9	373.9	170.8	112.0	8.6	4023.8
R07-2001	102.0	2.7	1.8	17.0	358.4	175.0	101.0	15.5	3752.6
Mean	99.0	2.4	1.7	17.4	366.2	172.9	107.2	11.9	3888.2
<u>Late</u>									
R07-2000	95.4	2.3	2.0	16.4	365.5	168.1	116.4	9.2	3438.7
R07-2001	96.9	2.7	1.7	16.2	349.1	177.9	117.6	11.2	3326.6
Mean	96.3	2.5	1.8	16.4	357.3	173.5	116.8	10.1	3378.0
<b>rs2</b>									
<u>Early</u>									
e3011	104.3	2.8	2.0	14.0	410.1	163.8	64.4	20.3	4017.1
IA2104HS	94.3	2.7	2.7	19.6	378.0	186.5	70.9	25.0	3564.3
IA3051HS	98.0	3.0	2.3	18.3	383.0	179.4	65.9	30.0	4207.6
CR13-399	127.3	4.0	1.8	16.8	373.6	193.5	48.5	24.9	2477.0
CR14-443	123.0	4.0	1.5	15.6	367.0	197.2	54.3	26.7	3519.4
Mean	109.4	3.2	2.1	16.8	382.3	184.1	60.8	25.4	3557.1
<u>Mid</u>									
e3011	110.7	3.3	1.7	14.4	410.0	164.7	62.8	19.3	4082.1
IA2104HS	94.0	2.5	2.0	19.9	378.6	183.6	65.9	22.2	3983.4
IA3051HS	101.0	2.7	1.7	19.1	385.5	179.8	65.7	28.6	4375.7
CR13-399	120.7	3.4	1.3	15.9	370.4	192.1	54.3	28.0	2694.5
CR14-443	113.3	3.0	1.3	15.0	355.9	198.3	55.5	26.4	3210.1
Mean	107.9	3.0	1.6	16.8	380.1	183.7	60.7	24.9	3669.2
<u>Late</u>									
e3011	85.3	3.8	1.3	15.6	409.5	166.4	64.1	19.1	2822.3
IA2104HS	77.9	3.4	1.3	18.3	371.4	184.4	76.6	25.2	2279.8

IA3051HS	77.9	4.1	2.0	18.8	372.7	180.8	64.8	25.4	2566.7
CR13-399	103.0	3.3	1.7	15.4	363.3	187.9	57.2	29.6	1681.3
CR14-443	103.3	3.3	1.3	14.6	353.3	193.9	60.1	26.8	2873.8
Mean	89.5	3.6	1.5	16.6	374.0	182.6	64.5	25.7	2447.8
<b>rs2/rs3</b>									
<u>Early</u>									
XD3553	103.0	2.7	1.8	14.8	389.9	167.8	64.2	21.3	4035.0
KB12-311027	117.3	3.8	2.2	12.9	393.5	176.8	68.5	18.1	3591.2
KB13-71102	126.7	3.4	1.8	14.2	375.3	189.3	73.5	20.1	3817.6
Mean	110.2	3.2	2.0	13.9	391.7	172.3	66.3	20.0	3768.7
<u>Mid</u>									
XD3553	115.7	2.2	1.5	14.5	393.5	162.6	68.5	21.7	4593.2
KB12-311027	123.0	4.0	2.7	13.7	398.6	172.1	66.7	17.7	3494.8
KB13-71102	126.7	2.8	1.7	13.7	363.6	189.4	72.9	24.2	4004.7
Mean	119.3	3.1	1.8	14.1	396.0	167.4	68.2	19.7	4044.0
<u>Late</u>									
XD3553	85.4	3.7	1.3	15.3	391.7	162.7	66.7	20.7	2811.1
KB12-311027	107.4	4.5	1.3	14.8	388.3	174.7	68.3	18.6	2427.7
KB13-71102	110.8	4.2	2.0	13.3	371.0	186.6	73.8	19.8	3192.1
Mean	96.8	4.1	1.3	15.1	390.0	168.9	67.8	19.4	2617.9
<b>LSD§</b>	3.7	0.3	0.2	0.5	5.1	3.4	3.5	2.2	225.4

† Type = type of genotype based on allelic mutation.

‡ Ht = height (cm); Lod = lodging score (1 = all plants erect, 5 = all plants down);

SDQT = seed quality score (1 = best, 5 = worst); SDWT = 100-seed weight (g);

Pro = protein concentration ( $\text{mg g}^{-1}$ ); Oil = oil concentration ( $\text{mg g}^{-1}$ ); Suc = sucrose concentration ( $\text{mg g}^{-1}$ ); RFOs = Raffinose Family of Oligosaccharides (raffinose + stachyose) ( $\text{mg g}^{-1}$ ); Yield = seed yield ( $\text{kg ha}^{-1}$ ).

§ LSD = least significant difference at the 0.05 probability level.

**Table 8.** Agronomic traits and seed composition of twenty genotypes as influenced by planting date (early, mid, late) at Novelty in 2016. Included are: ten specialty lines bred for an improved carbohydrate profile, and ten check cultivars.

2016 Novelty									
Type†	Ht‡	Lod	SDQT	SDWT	Pro	Oil	Suc	RFOs	Yield
<b>Check</b>									
Early	105.9	2.4	1.7	14.5	342.0	186.4	55.6	55.2	4390.6
Mid	93.8	2.1	1.5	15.5	343.1	186.1	59.4	55.3	4120.6
Late	61.9	1.4	1.7	15.1	349.0	176.6	62.4	53.4	2400.6
<b>mips1</b>									
<u>Early</u>									
R07-2000	99.3	2.7	2.0	14.2	358.0	171.2	109.0	8.3	1838.2
R07-2001	109.7	2.7	1.7	13.7	345.0	175.0	106.5	10.1	1981.6
Mean	104.5	2.7	1.9	14.4	351.7	172.7	107.8	9.2	1784.0
<u>Mid</u>									
R07-2000	89.3	1.8	1.8	14.2	353.8	166.5	117.0	8.5	2039.9
R07-2001	99.7	2.3	2.0	13.8	341.8	169.3	109.3	10.2	2147.5
Mean	94.5	2.1	1.9	14.0	347.6	168.2	114.1	9.2	2014.7
<u>Late</u>									
R07-2000	88.0	2.5	2.3	13.4	362.5	155.1	114.3	8.4	1869.6
R07-2001	84.0	1.5	1.7	13.1	353.8	155.8	109.3	12.6	2004.1
Mean	86.0	2.0	2.0	13.3	358.0	155.5	112.2	10.0	1927.5
<b>rs2</b>									
<u>Early</u>									
e3011	107.0	2.5	1.8	15.8	417.1	160.0	66.8	19.2	4227.8
IA2104HS	88.0	2.0	1.7	20.9	378.7	184.4	84.6	26.2	3658.4
IA3051HS	99.7	1.8	1.5	19.1	386.6	177.0	81.6	29.3	2640.7
CR13-399	116.3	2.3	2.0	16.8	366.9	188.1	58.1	23.7	2705.7
CR14-443	112.0	2.9	2.3	12.6	362.0	191.2	57.5	25.2	1748.6
Mean	104.6	2.3	1.9	17.1	382.3	180.3	69.0	24.8	3068.5
<u>Mid</u>									
e3011	97.0	1.7	1.5	16.4	413.6	161.9	74.8	19.4	3705.5
IA2104HS	80.0	1.5	1.5	20.3	379.1	179.3	72.0	19.7	3037.5
IA3051HS	83.7	1.5	1.2	19.6	385.4	175.7	79.5	30.2	2864.8
CR13-399	94.7	1.5	1.5	17.1	356.1	189.6	61.7	23.2	2266.4
CR14-443	100.0	2.7	1.8	16.2	352.7	193.1	58.8	22.6	2158.7
Mean	91.1	1.8	1.5	17.9	377.7	179.7	69.4	23.0	2825.8
<u>Late</u>									
e3011	60.7	1.3	1.5	17.2	430.0	150.1	74.0	18.6	2400.8
IA2104HS	57.0	1.0	2.0	18.6	381.4	168.0	88.2	22.1	2120.6

IA3051HS	61.3	1.3	1.3	18.5	386.0	165.7	80.6	27.3	2147.5
CR13-399	94.7	1.5	1.8	14.5	362.6	171.9	69.6	27.4	1562.5
CR14-443	75.0	1.5	1.8	13.0	355.3	179.2	69.2	23.6	1634.2
Mean	64.4	1.3	1.7	16.4	383.0	166.9	76.1	23.6	1988.1
<b>rs2/rs3</b>									
<u>Early</u>									
XD3553	108.7	1.5	1.7	17.3	392.8	160.1	77.5	22.4	5142.4
KB12-311027	119.3	3.2	1.7	13.5	396.4	173.8	83.4	22.0	3053.2
KB13-71102	127.3	3.4	1.7	13.4	369.5	186.0	74.0	22.3	3624.8
Mean	114.0	2.3	1.7	15.4	394.8	166.8	81.1	22.7	4159.1
<u>Mid</u>									
XD3553	90.7	1.6	1.5	17.2	402.2	159.5	76.3	22.4	4400.4
KB12-311027	102.7	3.0	1.2	15.0	399.6	169.9	81.3	18.1	3098.0
KB13-71102	108.0	2.8	1.7	14.9	366.1	186.8	66.3	16.8	3221.3
Mean	96.7	2.2	1.3	16.1	401.1	164.5	78.3	20.7	3834.4
<u>Late</u>									
XD3553	64.7	1.3	1.5	16.3	396.5	157.1	86.5	27.4	2790.9
KB12-311027	74.0	1.3	1.3	14.5	398.5	160.4	82.7	19.0	2300.0
KB13-71102	75.7	1.5	1.3	12.9	367.6	176.9	72.6	18.3	2078.0
Mean	69.3	1.3	1.4	15.4	397.3	158.8	84.6	23.2	2510.8
<b>LSD§</b>	3.7	0.2	0.2	0.8	6.1	3.3	4.0	2.6	321.8

† Type = type of genotype based on allelic mutation.

‡ Ht = height (cm); Lod = lodging score (1 = all plants erect, 5 = all plants down);

SDQT = seed quality score (1 = best, 5 = worst); SDWT = 100-seed weight (g);

Pro = protein concentration ( $\text{mg g}^{-1}$ ); Oil = oil concentration ( $\text{mg g}^{-1}$ ); Suc = sucrose concentration ( $\text{mg g}^{-1}$ ); RFOs = Raffinose Family of Oligosaccharides (raffinose + stachyose) ( $\text{mg g}^{-1}$ ); Yield = seed yield ( $\text{kg ha}^{-1}$ ).

§ LSD = least significant difference at the 0.05 probability level.

**Table 9.** Agronomic traits and seed composition of twenty genotypes as influenced by planting date (early, mid, late) at Portageville in 2016. Included are: ten specialty lines bred for an improved carbohydrate profile, and ten check cultivars.

2016 Portageville									
Type†	Ht‡	Lod	SDQT	SDWT	Pro	Oil	Suc	RFOs	Yield
<b>Check</b>									
Early	85.9	2.6	1.9	13.6	339.9	196.3	38.9	50.5	3909.4
Mid	95.3	2.7	1.8	13.4	339.4	198.6	42.8	53.4	3429.4
Late	98.3	2.9	2.0	12.9	351.1	192.1	39.0	55.4	3377.4
<b>mips1</b>									
<u>Early</u>									
R07-2000	60.6	2.0	1.7	14.2	377.0	180.6	81.4	8.9	3205.4
R07-2001	66.6	2.1	1.8	13.2	350.1	189.8	62.1	22.5	3208.8
Mean	64.2	2.0	1.8	13.7	366.8	185.5	71.9	15.7	3207.1
<u>Mid</u>									
R07-2000	93.9	2.3	2.0	13.0	375.4	178.3	82.8	9.0	2962.6
R07-2001	101.5	2.7	1.5	13.1	357.1	184.2	70.1	19.8	3105.2
Mean	99.4	2.5	1.8	13.1	364.7	182.2	76.5	14.4	3036.6
<u>Late</u>									
R07-2000	89.1	3.0	2.0	14.6	365.0	181.4	87.2	8.5	3099.7
R07-2001	89.6	3.0	2.0	13.8	361.0	185.4	78.9	14.8	2957.1
Mean	93.2	3.0	2.0	14.2	362.7	183.7	82.9	11.6	2988.3
<b>rs2</b>									
<u>Early</u>									
e3011	84.1	2.0	2.0	13.9	415.3	165.8	60.9	21.0	3725.6
IA2104HS	59.8	2.4	2.4	18.8	351.8	192.3	50.6	25.7	2282.7
IA3051HS	69.7	2.3	2.7	16.9	367.0	179.9	85.0	48.2	2244.8
CR13-399	90.5	3.0	2.0	13.4	353.4	203.8	38.7	26.8	1565.2
CR14-443	111.8	3.7	2.0	14.8	362.1	205.3	35.1	22.1	1945.4
Mean	85.1	2.7	2.2	15.5	370.3	189.3	51.3	28.1	2401.0
<u>Mid</u>									
e3011	89.7	2.7	2.0	13.6	401.7	169.7	54.7	20.0	3274.8
IA2104HS	73.7	2.3	1.7	17.8	356.9	197.7	86.7	37.3	2163.4
IA3051HS	91.6	3.0	1.8	16.7	360.0	188.3	94.1	46.8	2819.5
CR13-399	84.0	2.7	2.0	14.3	360.8	206.1	39.9	26.5	701.3
CR14-443	119.4	3.0	1.7	14.4	353.1	209.5	41.3	23.2	2288.0
Mean	92.0	2.7	1.8	15.4	366.8	193.6	56.6	28.2	2383.3
<u>Late</u>									
e3011	94.4	3.0	1.7	13.9	423.6	165.9	58.7	20.3	3292.0
IA2104HS	87.6	2.6	1.7	18.3	367.9	193.5	76.6	27.0	3078.9

IA3051HS	89.2	3.0	2.0	18.2	381.3	186.5	63.1	29.4	3132.5
CR13-399	114.1	3.0	2.0	14.1	374.5	198.4	41.1	26.3	2066.0
CR14-443	106.0	4.0	1.8	14.0	373.2	204.7	42.9	22.8	1751.5
Mean	98.4	3.1	1.8	15.7	384.7	189.2	56.5	25.1	2704.6
<b>rs2/rs3</b>									
<u>Early</u>									
XD3553	89.1	2.3	1.8	14.8	389.0	165.6	70.8	24.3	4337.4
KB12-311027	103.8	3.0	2.2	13.3	378.4	183.5	70.9	21.6	2997.7
KB13-71102	112.3	3.0	1.8	12.3	364.3	198.2	50.6	17.5	2218.4
Mean	96.1	2.7	2.0	14.0	383.4	173.3	70.8	22.9	3662.7
<u>Mid</u>									
XD3553	86.3	2.3	1.5	14.6	388.9	168.2	75.9	26.3	3658.1
KB12-311027	122.6	3.0	1.3	13.5	378.0	186.3	62.2	17.0	3122.0
KB13-71102	116.5	3.0	2.0	13.5	353.1	202.5	53.7	22.8	1691.0
Mean	105.1	2.7	1.4	14.0	381.6	176.5	67.6	20.8	3391.3
<u>Late</u>									
XD3553	83.7	3.0	2.0	13.9	411.8	161.7	83.0	30.9	3512.8
KB12-311027	110.3	4.0	1.3	14.3	401.1	183.5	59.2	17.0	3132.5
KB13-71102	123.7	3.0	2.2	12.7	377.5	194.0	53.8	20.2	2286.8
Mean	97.0	3.5	1.8	14.1	407.0	172.2	71.1	23.9	3393.0
<b>LSD§</b>	6.0	0.2	0.2	0.6	6.2	4.0	5.4	3.7	228.1

† Type = type of genotype based on allelic mutation.

‡ Ht = height (cm); Lod = lodging score (1 = all plants erect, 5 = all plants down);

SDQT = seed quality score (1 = best, 5 = worst); SDWT = 100-seed weight (g);

Pro = protein concentration ( $\text{mg g}^{-1}$ ); Oil = oil concentration ( $\text{mg g}^{-1}$ ); Suc = sucrose concentration ( $\text{mg g}^{-1}$ ); RFOs = Raffinose Family of Oligosaccharides (raffinose + stachyose) ( $\text{mg g}^{-1}$ ); Yield = seed yield ( $\text{kg ha}^{-1}$ ).

§ LSD = least significant difference at the 0.05 probability level.

**Table 10.** Pearson correlation coefficients between agronomic traits and seed composition constituents of twenty-one genotypes grown in 2015 and 2016 at the first (early) planting date. Included are: eleven specialty lines bred for an improved carbohydrate profile, and ten checks.

	<b>Ht§</b>	<b>Lod</b>	<b>Mat</b>	<b>SDQT</b>	<b>SDWT</b>	<b>Oil</b>	<b>Pro</b>	<b>Suc</b>	<b>RFOs</b>	<b>Yld</b>
<b>Ht</b>	-	0.86***	NS	-0.82***	NS	NS	NS	NS	NS	NS
<b>Lod</b>		-	NS	-0.67***	NS	NS	NS	NS	NS	NS
<b>Mat</b>			-	NS	NS	NS	NS	NS	NS	NS
<b>SDQT</b>				-	0.54*	NS	NS	NS	NS	-NS
<b>SDWT</b>					-	NS	NS	NS	NS	NS
<b>Oil</b>						-	-0.65**	-0.63**	0.51*	NS
<b>Pro</b>							-	NS	-0.74***	-0.49*
<b>Suc</b>								-	-0.71***	NS
<b>RFOs</b>									-	0.69***
<b>Yld</b>										-

§ Ht = height (cm); Lod = lodging score (1 = all plants erect, 5 = all plants down); Mat = maturity (days after September 1); SDQT = seed quality score (1 = best, 5 = worst); SDWT = 100-seed weight (g); Pro = protein concentration ( $\text{mg g}^{-1}$ ); Oil = oil concentration ( $\text{mg g}^{-1}$ ); Suc = sucrose concentration ( $\text{mg g}^{-1}$ ); RFOs = Raffinose Family of Oligosaccharides (raffinose + stachyose) ( $\text{mg g}^{-1}$ ); Yield = seed yield ( $\text{kg ha}^{-1}$ ).

\* significant at the 0.05 probability level.

\*\* significant at the 0.01 probability level.

\*\*\* significant at the 0.001 probability level.

**Table 11.** Pearson correlation coefficients between agronomic traits and seed composition constituents of twenty-one genotypes grown in 2015 and 2016 at the second (mid) planting date. Included are: eleven specialty lines bred for an improved carbohydrate profile, and ten checks.

	<b>Ht§</b>	<b>Lod</b>	<b>Mat</b>	<b>SDQT</b>	<b>SDWT</b>	<b>Oil</b>	<b>Pro</b>	<b>Suc</b>	<b>RFOs</b>	<b>Yld</b>
<b>Ht</b>	-	0.79***	NS	-0.44*	NS	NS	NS	NS	NS	NS
<b>Lod</b>		-	NS	NS	NS	NS	NS	NS	NS	NS
<b>Mat</b>			-	NS	-0.53*	NS	NS	NS	NS	NS
<b>SDQT</b>				-	NS	NS	NS	NS	NS	NS
<b>SDWT</b>					-	NS	NS	NS	NS	NS
<b>Oil</b>						-	-0.69***	-0.65**	0.45*	NS
<b>Pro</b>							-	NS	-0.71***	NS
<b>Suc</b>								-	-0.66**	NS
<b>RFOs</b>									-	0.71***
<b>Yld</b>										-

§ Ht = height (cm); Lod = lodging score (1 = all plants erect, 5 = all plants down); Mat = maturity (days after September 1); SDQT = seed quality score (1 = best, 5 = worst); SDWT = 100-seed weight (g); Pro = protein concentration ( $\text{mg g}^{-1}$ ); Oil = oil concentration ( $\text{mg g}^{-1}$ ); Suc = sucrose concentration ( $\text{mg g}^{-1}$ ); RFOs = Raffinose Family of Oligosaccharides (raffinose + stachyose) ( $\text{mg g}^{-1}$ ); Yield = seed yield ( $\text{kg ha}^{-1}$ ).

\* significant at the 0.05 probability level.

\*\* significant at the 0.01 probability level.

\*\*\* significant at the 0.001 probability level.

**Table 12.** Pearson correlation coefficients between agronomic traits and seed composition constituents of twenty-one genotypes grown in 2015 and 2016 at the third (late) planting date. Included are: eleven specialty lines bred for an improved carbohydrate profile, and ten checks.

	<b>Ht§</b>	<b>Lod</b>	<b>Mat</b>	<b>SDQT</b>	<b>SDWT</b>	<b>Oil</b>	<b>Pro</b>	<b>Suc</b>	<b>RFOs</b>	<b>Yld</b>
<b>Ht</b>	-	0.61**	0.44*	NS	-0.54*	NS	NS	NS	NS	NS
<b>Lod</b>		-	NS	-0.60**	NS	NS	NS	NS	NS	NS
<b>Mat</b>			-	0.54*	-0.48*	NS	NS	NS	NS	0.52*
<b>SDQT</b>				-	NS	NS	NS	NS	NS	NS
<b>SDWT</b>					-	NS	NS	NS	NS	NS
<b>Oil</b>						-	-0.64**	-0.66**	0.50*	NS
<b>Pro</b>							-	NS	-0.66**	NS
<b>Suc</b>								-	-0.73***	NS
<b>RFOs</b>									-	0.59**
<b>Yld</b>										-

§ Ht = height (cm); Lod = lodging score (1 = all plants erect, 5 = all plants down); Mat = maturity (days after September 1); SDQT = seed quality score (1 = best, 5 = worst); SDWT = 100-seed weight (g); Pro = protein concentration ( $\text{mg g}^{-1}$ ); Oil = oil concentration ( $\text{mg g}^{-1}$ ); Suc = sucrose concentration ( $\text{mg g}^{-1}$ ); RFOs = Raffinose Family of Oligosaccharides (raffinose + stachyose) ( $\text{mg g}^{-1}$ ); Yield = seed yield ( $\text{kg ha}^{-1}$ ).

\* significant at the 0.05 probability level.

\*\* significant at the 0.01 probability level.

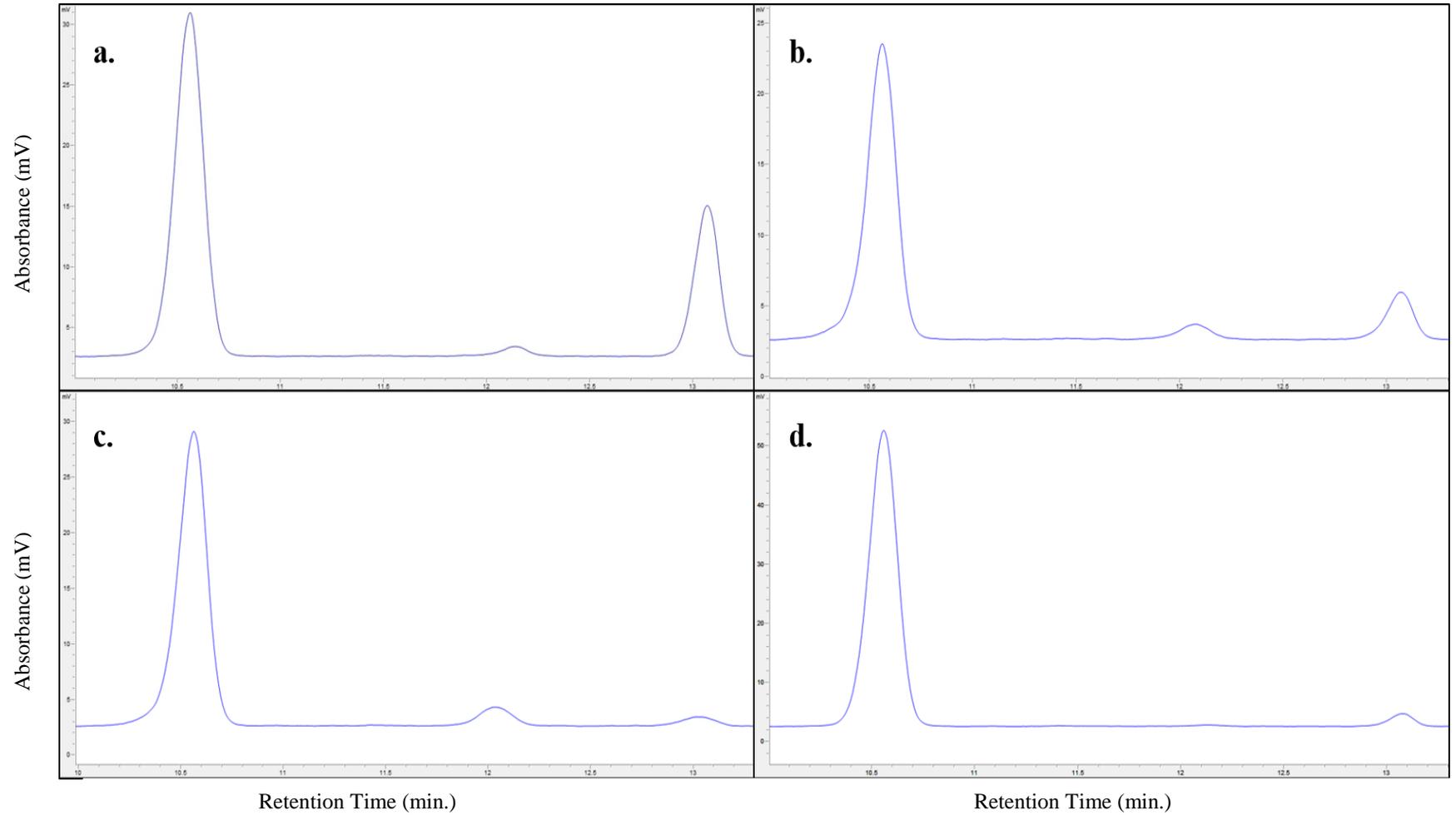
\*\*\* significant at the 0.001 probability level.

## Figures

**Figure 1.** Chromatograph examples acquired via HPLC of each genotypic class evaluated in this study. First peak = sucrose; Second peak = raffinose; Third peak = stachyose.

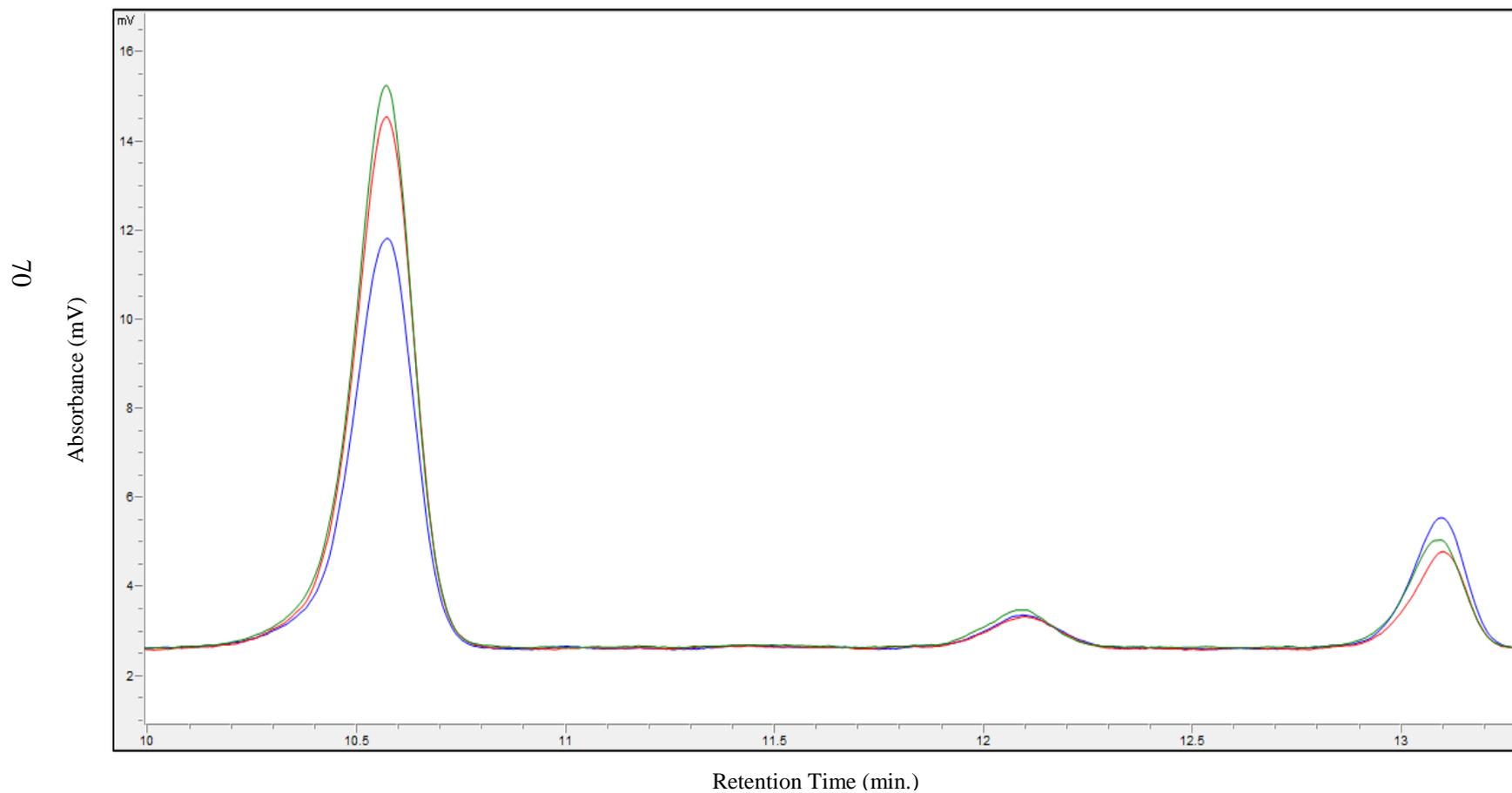
a = check; b = rs2; c = rs2/rs3; d = mips1

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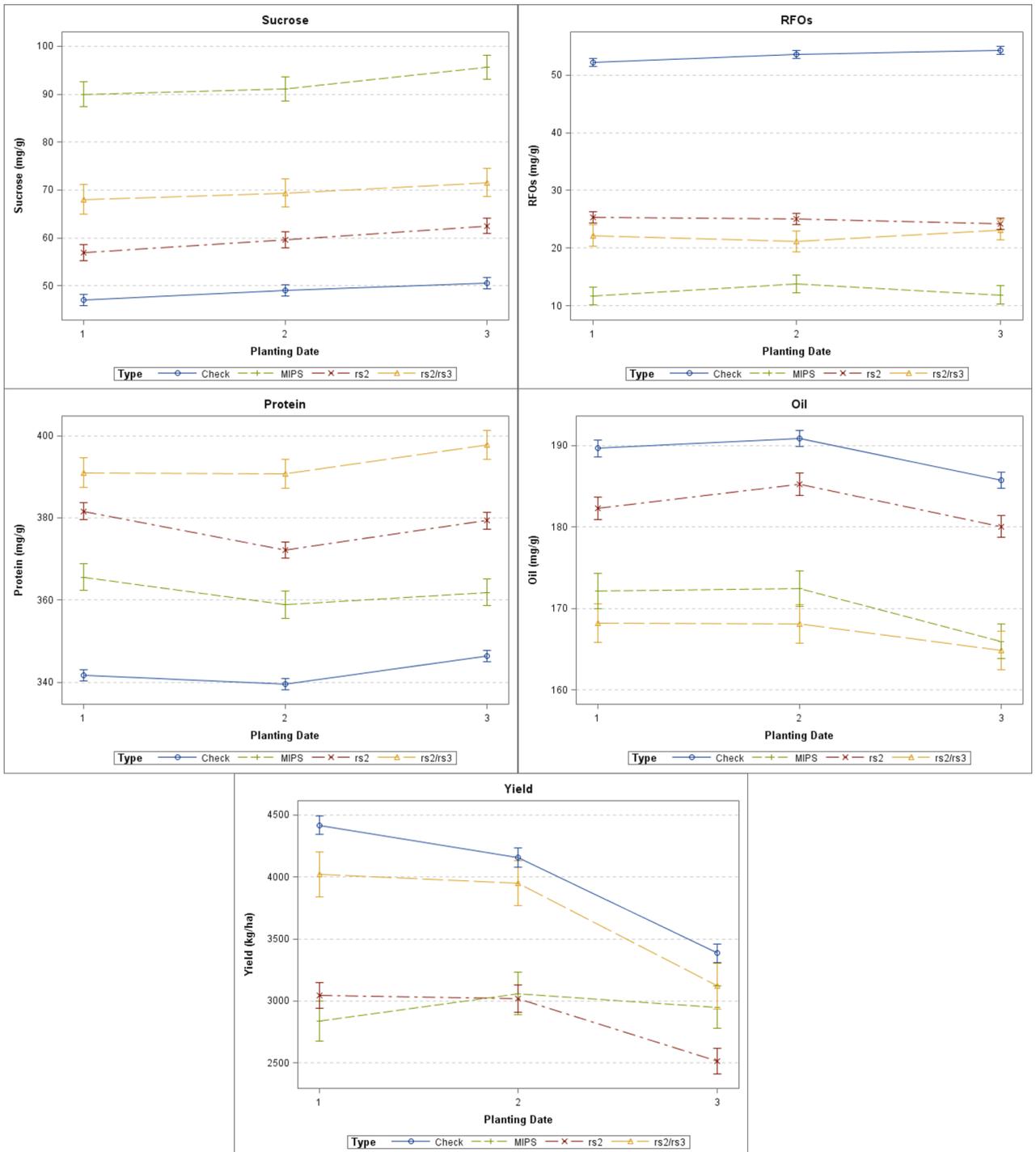
**Figure 2.** Chromatograph example acquired via HPLC of different carbohydrate concentrations at each planting date.

First peak = sucrose; Second peak = raffinose; Third peak = stachyose. The blue line represents the first planting date, the red line represents the second planting date, and the green line represents the third planting date.

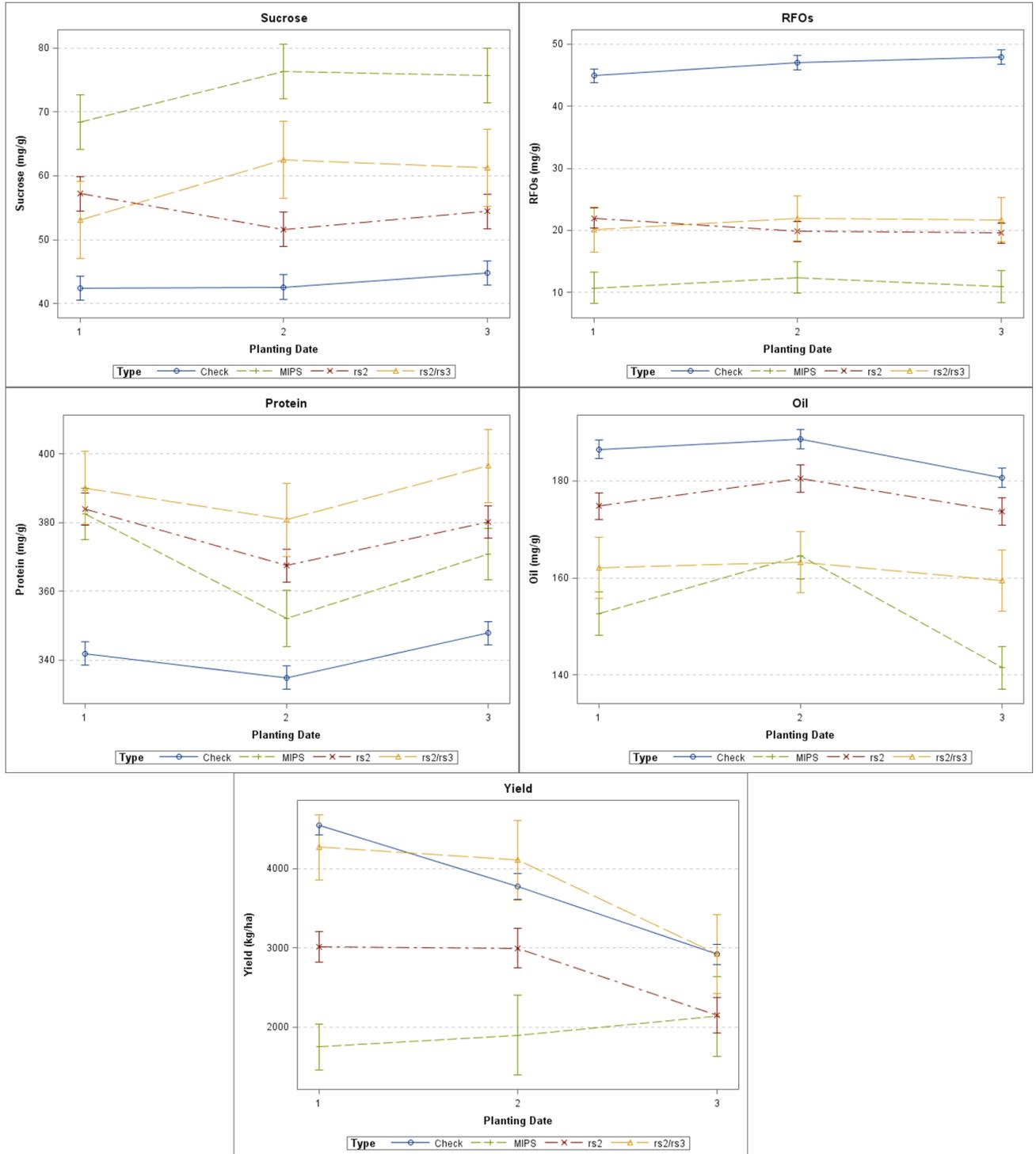


**Figure 3.** Planting date [1(early), 2(mid), 3(late)] effects on soybean yield and seed composition of twenty genotypes grown in 2015 and 2016, averaged across locations.

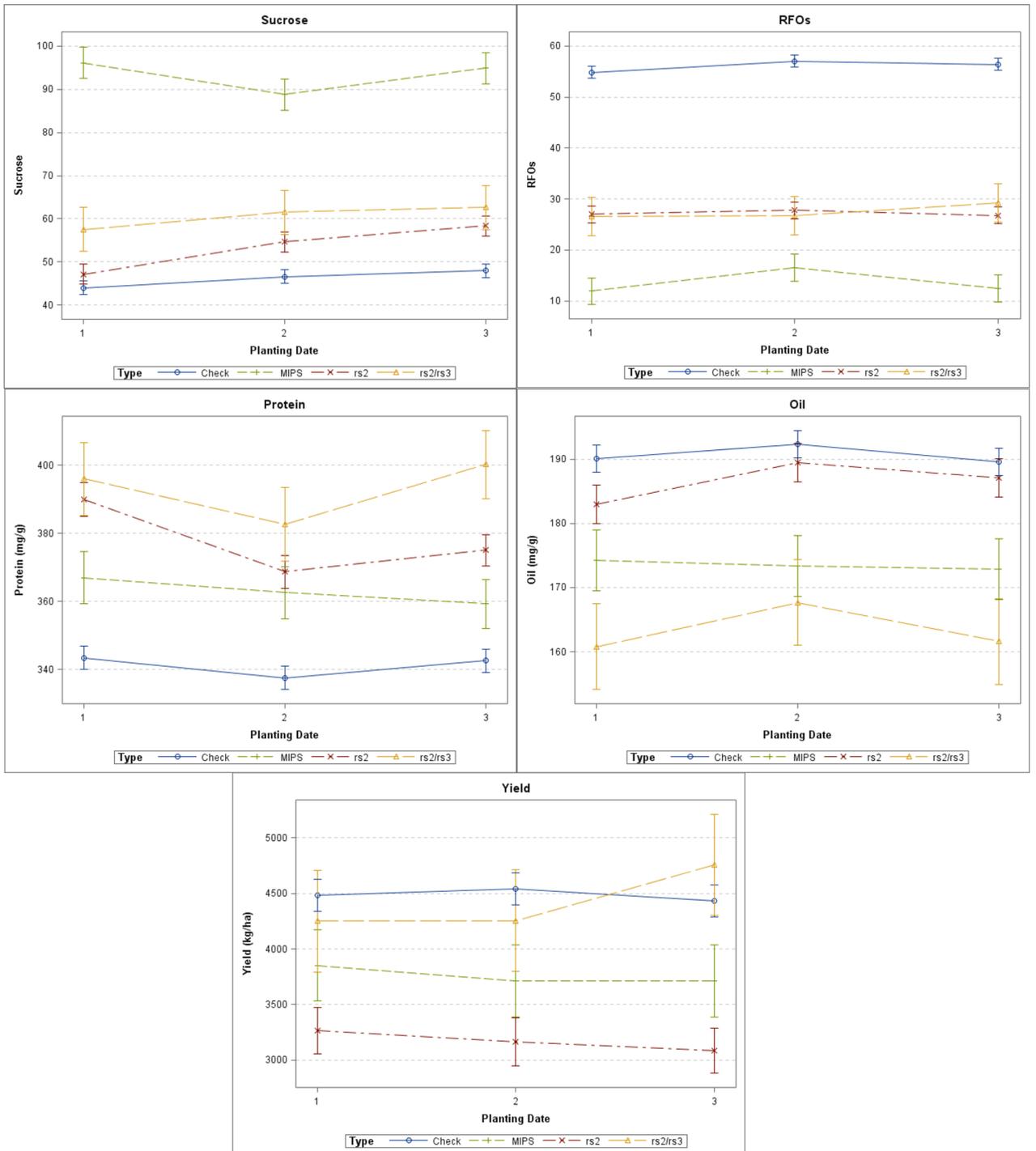
Included are: ten specialty lines bred for an improved carbohydrate profile, and ten check cultivars.



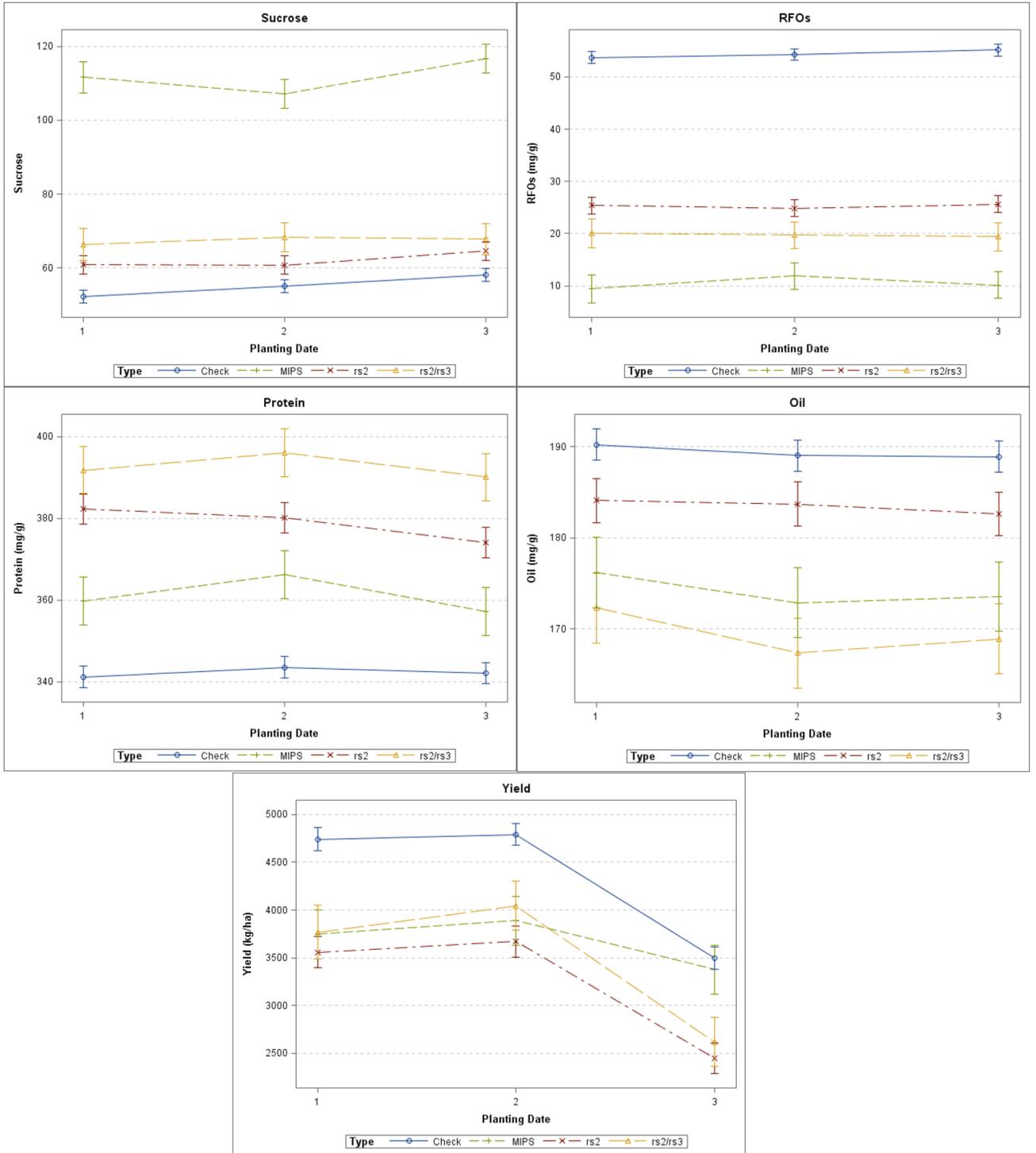
**Figure 4.** Planting date [1(early), 2(mid), 3(late)] effects on soybean yield and seed composition of eighteen genotypes grown in 2015 at the Columbia location. Included are: eight specialty lines bred for an improved carbohydrate profile, and ten check cultivars.



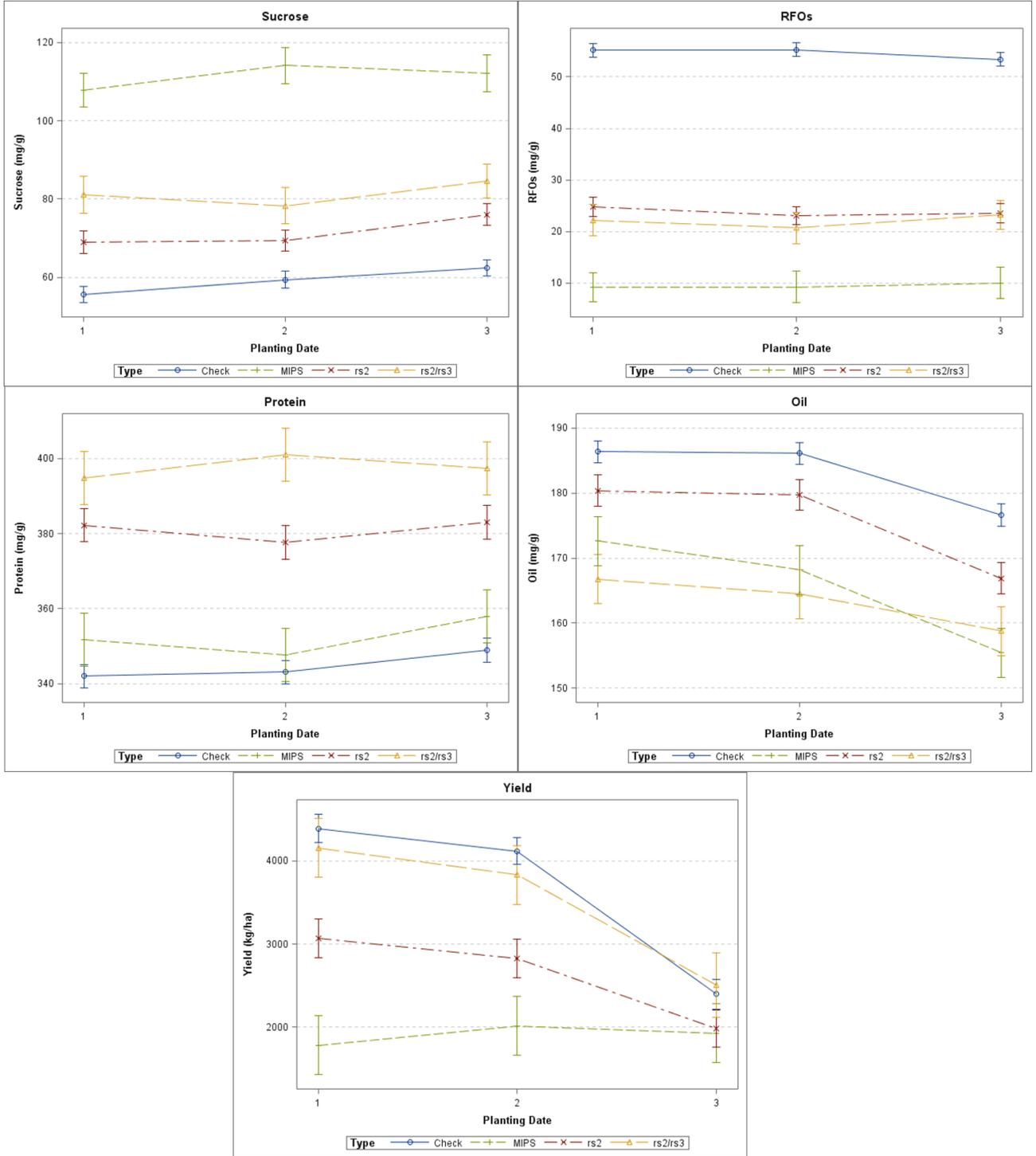
**Figure 5.** Planting date [1(early), 2(mid), 3(late)] effects on soybean yield and seed composition of eighteen genotypes grown in 2015 at the Portageville location. Included are: eight specialty lines bred for an improved carbohydrate profile, and ten check cultivars.



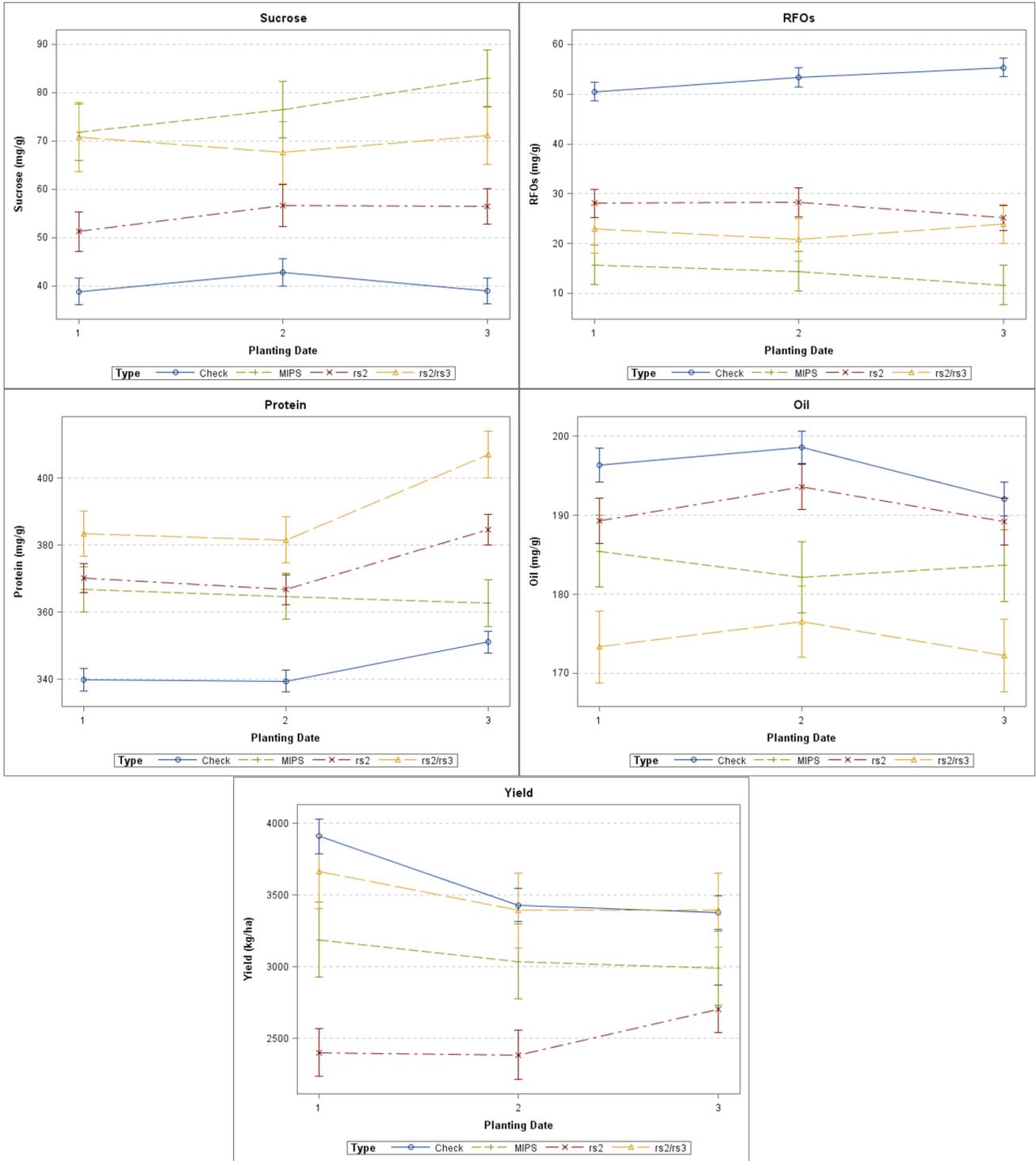
**Figure 6.** Planting date [1(early), 2(mid), 3(late)] effects on soybean yield and seed composition of twenty genotypes grown in 2016 at the Columbia location. Included are: ten specialty lines bred for an improved carbohydrate profile, and ten check cultivars.



**Figure 7.** Planting date [1(early), 2(mid), 3(late)] effects on soybean yield and seed composition of twenty genotypes grown in 2016 at the Novelty location. Included are: ten specialty lines bred for an improved carbohydrate profile, and ten check cultivars.



**Figure 8.** Planting date [1(early), 2(mid), 3(late)] effects on soybean yield and seed composition of twenty genotypes grown in 2016 at the Portageville location. Included are: ten specialty lines bred for an improved carbohydrate profile, and ten check cultivars.



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## **Chapter III**

# **Economic Impact of Soybeans with Modified Carbohydrate Profiles**

## **Abstract**

Information on management practices that can increase crop value can be useful for producers in maximizing farm profits. Seed composition and yield have been shown to be affected by differing planting dates, which alters the environment in which a crop is grown. Providing soybean meal high in sucrose and low in Raffinose Family of Oligosaccharides (RFOs) is desirable for improving digestibility and increased feed efficiency in monogastric animals. Soybean meal with high sucrose and low RFO concentrations has the potential to increase metabolizable energy, and soybean producers are able to receive a premium for feed that meets these standards. In this study, the economic impact of specialty soybean genotypes were investigated to determine if it is economically feasible to grow these genotypes for specialty markets as opposed to growing commodity type soybeans. Three models were created to simulate different levels of premiums for high sucrose/low RFO soybeans, at \$0.50, \$0.75, and \$1.00 per bushel. The experiment was arranged in a split-plot design, with planting date as the main plot, and genotype as the subplot, performed in 2015 and 2016 at three locations each year in Missouri, each with three planting dates. Planting dates were chosen to simulate early, full season, and double crop conditions. Planting date had a significant effect on seed yield ( $p < 0.001$ ) and sucrose ( $p < 0.01$ ). Early plantings showed increased yield, while late plantings showed increased sucrose. At the \$0.50 premium level, the late planting of the rs2/rs3 group showed positive gross returns when compared to the late planted checks. At the \$0.75 premium level, the rs2/rs3 group across all planting dates showed positive gross returns when compared to the checks. At the \$1.00 premium level, the rs2/rs3 group across all plantings showed positive gross returns when compared to the checks, in upwards of \$35 per acre. This research

shows that growing soybeans with the high sucrose/low RFO trait for specialty markets have the ability to improve profitability for soybean producers, and they will fit best in a double-cropping production system in Missouri. These results will provide useful information to soybean producers aiming to grow these types of specialty soybeans for niche markets in aims of increasing farm profits.

## **Introduction**

Soybean [*Glycine max* (L). Merrill] is one of the most important economic crops grown in the United States, and is the leading oilseed crop produced and consumed in the world (Wilcox, 2004). Soybean seeds consist of 40% protein, 20% oil, 35% carbohydrates, and 5% ash, on a dry-weight basis (Hymowitz and Collins, 1974).

Soybean meal is an important component of livestock feed, particularly for monogastric animals, due to its high protein content (~47%) and for being an excellent source of dietary fiber. In 2015, over 30 million metric tons of soybean meal were used for livestock feed, with poultry and swine using 16.3 (54%) and 6.9 (23%) million metric tons, respectively (ASA, 2016). The three major soluble carbohydrates in soybean include sucrose, raffinose, and stachyose (Karr-Lilienthal et al., 2005); (Liu, 1997). The concentration of the soluble carbohydrates can play a role in determining the usefulness of soybean meal. Of the three major components, only sucrose is nutritionally beneficial and easily digested to be used as metabolizable energy for monogastric animals.

Alternatively, the raffinose family of oligosaccharides (RFOs), primarily comprised of raffinose and stachyose, are considered as anti-nutritional factors in soybean meal because they are unable to be digested by monogastric animals due to the lack of the enzyme  $\alpha$ -galactosidase in the stomach and small intestine (Sebastian, 2000). The poor

digestion of RFOs can lead to a reduction in metabolizable energy, ultimately resulting in poor weight gain and substantial loss in market value in livestock production operations (Rackis, 1974); (Zuo et al., 1996). As soybean seeds are processed into soybean meal, several steps of soaking, heating, and pressure treatments are used. These processing steps decrease both RFO and sucrose concentrations, but still leave undesirable amounts of RFOs in the resulting soybean meal (Leske and Coon, 1999). More sophisticated processing and extraction methods are available to reduce RFOs, but they are currently not economically practical for use in large scale livestock feed production systems. Thus, utilizing genetic and environmental means to reduce RFOs in soybean seeds is desirable for producing high sucrose, low RFO soybean meal to be used in livestock feed.

Genetic control offers a solution to removing unwanted RFOs in soybean seeds (Deak et al., 2006). A soybean plant introduction, PI200508, was shown to have reduced levels of RFOs and increased sucrose, which was found to be due to reduced raffinose synthase activity controlled by the mutant allele *stc1* (Sebastian, 2000). Soybean raffinose synthase activity has been studied by Hitz et al. (2002) and Dierking and Bilyeu (2008). These studies have shown that the soybean genome contains at least two expressed genes similar to other characterized raffinose synthases in other plants, raffinose synthase 2 (RS2) and raffinose synthase 3 (RS3), respectively. The novel allele of RS2 from PI200508 has been shown to completely associate with the low RFO phenotype. RS3 is also thought to contribute to the low RFO phenotype, but to a lesser degree as compared to the RS2 allele (Dierking and Bilyeu, 2008). The soybean line LR33 was shown to have reduced levels of RFOs, as well as an increase in sucrose, due to a novel allele of the myo-inositol phosphate-1 synthase gene (MIPS1), which plays an

important role in the biosynthesis of raffinose and stachyose (Hitz et al., 2002) (Kerr et al., 2003).

Planting date is a management practice that has been shown to affect soybean growth and development, seed yield, and seed composition. The environment in which a crop is grown can be manipulated by changing the planting date, and this in turn changes the range of temperatures, soil moisture levels, and day lengths that a crop is exposed to. Previous research has shown that for genotypes of the same maturity group, the later that soybeans are planted, the more likely the seed-fill period will take place in cooler temperatures than earlier planted soybeans, which can significantly affect seed composition. Previous research has shown that delaying soybean planting led to significant increases in sucrose concentrations (Bellaloui, 2011); (Jaureguy et al., 2013). Hagely et al. (2013) evaluated soybean lines with different combinations of alleles of the RS2 gene in one location. This study indicated variability between carbohydrate profiles of different genotypes, but showed that each genotypic class had a distinct carbohydrate profile. Another study, conducted by Bilyeu and Wiebold (2016), evaluated soybean lines with different combinations of mutant RS2 and RS3 alleles at different planting dates. This study showed significant increases in sucrose concentrations with later planting dates, and suggested that higher sucrose concentrations are produced with cooler temperatures during the seed-fill period.

For livestock producers, feed rations are specifically formulated to specific nutrient requirements on a least-cost basis. Soybean meal with high sucrose and low RFO concentrations has the potential to increase metabolizable energy by upwards of seven to nine percent (Parsons et al., 2000), and industry participants would be willing to pay a

premium for feed that increases metabolizable energy. Since soybean meal provides a source of protein to monogastric animals, it could replace animal-derived protein sources that are currently supplemented due to soybean meal being significantly cheaper. It has been estimated that the potential premium that soybean producers could gain from growing high sucrose/low RFO soybean meal would range from \$0.50 to \$1.00 per bushel (Parcell et al., 2014; Shivley and Parcell, 2014), in an identity preserved system. Since most premiums are paid on a per bushel basis, producers must take into account the ability of a cultivar to yield well within their selected environment in order to maximize profits gained from growing soybeans for a specialty market in aims of receiving a premium. Previous research on the economic impacts of soybean quality traits is limited. Helms et al., 1990 investigated the economic feasibility of delaying planting in order to increase protein content in order to receive a premium. This study showed that the increased value due to higher protein concentrations did not economically compensate for the decreased seed yield from delaying planting. More research is needed to evaluate these specialty soybean genotypes to determine whether it will be more profitable to grow high sucrose/low RFO soybeans for specialty markets as opposed to commodity type soybeans.

The objectives of this study are to determine the economic impact of utilizing soybean genotypes with a modified carbohydrate profile consisting of high sucrose and low RFO concentrations. This research aims to determine if these specialty soybean genotypes with a modified carbohydrate profile will lead to an increased net return for producers when compared to a traditional full-season soybean crop.

## Materials and Methods

### Field Experiment

Eighteen genotypes were grown in 2015 (Table 1); eight specialty lines bred for an improved carbohydrate profile, and ten check cultivars. Twenty genotypes were grown in 2016 (Table 2); ten specialty lines bred for an improved carbohydrate profile, and ten check cultivars. Genotypes were divided by maturity group (III and IV). The experiment was arranged in a split-plot design, with planting date as the main plot, and genotype as the subplot. This study was planted at three locations in Missouri during 2015 and 2016: the Bradford Research and Extension Center (REC) near Columbia (N38°53'36.80", W92°12'53.21), the Greenley Memorial REC near Novelty (N40°01'18.6", W92°11'25.3") and the Lee Farm REC near Portageville (N36°23'43.7", W89°36'56.5"). Soil types are as follows: Bradford REC – Mexico silt loam (fine, smectitic, mesic, Aeric Vertic Epiaqualfs); Greenley Memorial REC – Putnam silt loam (fine, smectitic, mesic Vertic Albaqualfs); Lee Farm REC – Tiptonville silt loam (fine-silty, mixed, superactive, thermic Oxyaquic Argiudolls). Soybeans were rotated with corn (*Zea mays* L.) in 2015 and 2016 at all locations. Plots were planted with an ALMACO cone planter (ALMACO Inc. Nevada, IA) with four rows spaced at 0.76m. Plot dimensions were 4.88m by 2.29m. Planting dates were early May (early), late May/early June (mid), and late June/early July (late). Planting dates in 2015 were May 6, June 10, and June 24 for Columbia; June 5 and July 14 for Novelty; May 13, May 29, and June 16 for Portageville. Planting dates in 2016 were May 5, May 25, and June 15 for Columbia; May 24, June 15, and July 19 for Novelty; May 16, June 1, and June 8 for Portageville. In 2015, the early Novelty planting date was lost due to excessive rainfall and pest pressure. The center two rows of each plot

were harvested at maturity using an ALMACO SPC-40 plot combine (ALMACO, Inc. Nevada, IA).

### **Seed Composition Analysis**

*Carbohydrate analysis:* Soluble carbohydrates sucrose, raffinose, and stachyose were quantified by high performance liquid chromatography (HPLC) equipped with an evaporative light scattering detection (ELSD) system, as described by Valliyodan et al. (2015), with slight modifications. Around 10 seeds from each plot were randomly selected and ground, and the ground powder was lyophilized for 48 hours in a Labconco Freeze Dry System (Labconco, USA). Soluble carbohydrates of the seed were extracted by an acetonitrile extraction method. Briefly, about 0.09 g (0.0895-0.0905 g) of the dried sample was weighed and mixed with 900  $\mu$ L HPLC grade water in 2 mL centrifuge vials. The vials were incubated at 55°C for 30 minutes at 200 rpm agitation, followed by 20 seconds of high speed vortex. 900  $\mu$ L of 95% HPLC grade acetonitrile was then blended in, with an additional 20 seconds of high speed vortex. Samples were then centrifuged at 10000 g for 10 minutes. 800  $\mu$ L of the supernatant was then filtered through a 0.45  $\mu$ m filter. 100  $\mu$ L of the sample solution was finally mixed with 400  $\mu$ L of 65% HPLC grade acetonitrile in a 2 mL glass HPLC vial. Sugar standards for sucrose, raffinose, and stachyose from Sigma-Aldrich (St. Louis, MO) were prepared in water with concentrations of 50, 100, 300, 500, and 1000  $\mu$ g/mL. The HPLC system used for this experiment was an Agilent 1200 series (Agilent, USA). The separation of the soluble carbohydrates was performed by a Prevail Carbohydrate ES column (5  $\mu$  250 mm x 4.6 mm), coupled to a guard column (7.5 x 4.6 mm), from Grace Davison Discovery Sciences (Deerfield, IL). Two mobile phases were prepared: mobile phase A was 100%

HPLC grade water, and mobile phase B was an acetonitrile: acetone mixture of 75:25 (v/v), with a flow rate of 1.2 mL/min. Column temperature was maintained at 35°C, and the detector temperature was isothermal at 55°C. Nebulizer pressure was maintained at 3.4 bar, with ultrapurity-grade nitrogen used as the nebulizer gas. Sample injections were made at a volume of 5 µL. Sample run time was a total of 18 minutes, with the first 14 minutes for sample separation followed by a 4 minute reconditioning step. Sucrose, raffinose, and stachyose were quantified based on standard curves generated for each carbohydrate. Concentrations of sucrose, raffinose, and stachyose are presented in milligrams of sugar per gram of seed ( $\text{mg g}^{-1}$ ).

### **Statistical Analysis**

Based on the combination of alleles, genotypes were grouped into four different categories, referred to as ‘types’: genotypes containing a mutant RS2 allele (rs2), genotypes containing a combination of mutant RS2 and RS3 alleles (rs2/rs3), genotypes containing the mutant MIPS1 allele (mips1), and genotypes containing wild type alleles (checks). The combination of years and locations were considered as five different environments included in the statistical analysis. This experiment was analyzed as a split-split plot, with environment as the whole plot, planting date as the split-plot, and genotype as the split-split plot. All factors were considered fixed in the analysis, except for plot within location (plot (location)), and plot within location at each planting date (plot (location planting date)). Analysis of variance (ANOVA) was conducted over all environments using PROC GLIMMIX procedure in SAS 9.3 (SAS Institute, 2011). Means were estimated using the lsmeans option, and significant differences between the least squares means (LS-means) were computed by using the "lines" option.

## **Economic Analysis**

Gross value per acre was calculated by producing three models, each simulating a different premium rate for high sucrose/low RFO soybeans; at \$0.50, \$0.75, and \$1.00 per bushel added on top of the current market value per bushel for commodity soybeans. The current market value of soybean used was \$9.60 per bushel, as of 3/31/17 (CME Group, 2017). Each genotypic type used in this study was compared to the check cultivars across each maturity group at each corresponding planting date (Helms et al., 1990).

## **Results and Discussion**

### **Planting Date Effects on Seed Carbohydrates and Yield**

Analysis of variance was conducted to identify environment, planting date, and type effects on all seed carbohydrates (sucrose and RFOs) and yield. The overall ANOVA showed that the environment (Env) effect was significant on all seed carbohydrates and yield (Table 3). The planting date (PD) effect was significant on sucrose and yield, but not significant on raffinose, stachyose, or combined RFOs. The type (T) effect was significant on all seed carbohydrates and yield. The significant variance between genotypic groups was expected, due to this study containing both commodity type cultivars, and specialty lines bred for an improved carbohydrate profile. The Env x T effect was significant on all seed carbohydrates and yield. The PD x T effect was significant on raffinose, stachyose, and yield (Table 3). There were no other significant effects.

Overall, sucrose was significantly higher in late plantings as compared to earlier plantings. Yields were significantly higher in the early planting dates as compared to the late plantings. Concentrations of RFOs were not significantly different at any of the three planting dates. These results are consistent with previous research on planting date effects on soybean carbohydrate profiles (Bellaloui, 2011); (Jaureguy et al., 2013); (Bilyeu and Wiebold, 2016), and yield (Egli and Cornelius, 2009); (Chen and Wiatrak, 2010); (Hu and Wiatrak, 2012; Kandel et al., 2016; Marburger et al., 2016; Salmerón et al., 2016). Within genotypic types, these trends generally stayed the same. For all seed carbohydrates and yield, each genotypic type was significantly different from the others. The checks group had significantly higher yields overall compared to the other groups, with the rs2/rs3 group having significantly higher yields than the mips1 and rs2 groups. For sucrose, the mips1 group had the highest concentrations, followed by: rs2/rs3, rs2, and checks, respectively. For RFOs, the checks had the highest concentrations, followed by: rs2, rs2/rs3, and mips1, respectively.

These results indicate that the accumulation of carbohydrate components is primarily genotype dependent, with environmental factors also influencing the accumulation of sucrose. Planting date also showed a significant effect on sucrose concentrations and yield, with the late planting having significantly higher sucrose concentrations than both the early and mid-planting. These trends were true for all genotypic types, thus showing that later planting dates will favor both commodity-type and specialty genotypes for the production of high sucrose soybean meal to meet specialty market demands. The mips1 group exhibited the most advantageous carbohydrate profile to meet specialty market demands, by having the highest sucrose

concentrations overall, and also having the lowest concentrations of RFOs. The rs2/rs3 group had the second highest concentration of sucrose and the second lowest concentrations of RFOs, both significantly better than the group with only the rs2 mutant allele, and all exhibiting a significantly improved carbohydrate profile as compared to the check group.

### **Weather Data Associations with Seed Composition Components**

Based on previous research, temperature during the pod-filling period is the primary environmental factor affecting seed composition (Kane et al., 1997; Piper and Boote, 1999; Wolf et al., 1982). During the two years of this study, 2015 had lower temperatures overall, and during August – October, during which the pod-filling period took place across the varying planting dates (Table 2). Location years were considered as separate locations for this study. The Portageville locations showed the highest temperatures overall, followed by the Columbia and Novelty locations, respectively. These temperature trends were consistent with many observed trends for the seed composition components measured. Overall, sucrose concentrations were higher at the Columbia and Novelty locations (Tables 5, 7, 8) as compared to the Portageville locations (Tables 6, 9). This reinforces previous research showing cooler temperatures during pod-fill associating with sucrose accumulation (Bellaloui, 2011); (Jaureguy et al., 2013); (Bilyeu and Wiebold, 2016). Conversely, RFO concentrations were highest at the Portageville locations, where temperatures were highest. This information suggests that producing high sucrose soybeans is most easily attained through delaying planting in order to ensure that the pod-filling period occurs during lower temperatures.

## Economic Analysis

Overall, total value per acre increased as planting date was delayed across all models. Yield decreased with later plantings, and sucrose increased with later plantings, while RFO concentrations were not significantly affected by planting date. In the model with a \$0.50 premium per bushel, the late planting of the rs2/rs3 group yielded a positive gross return when compared to the late planted check soybean check (\$11.62/acre) (Table 10) (Figure 1). There were no other positive gross returns among the specialty groups at any planting date.

In the \$0.75 premium per bushel model, each planting of the rs2/rs3 type yielded a positive gross return when compared to the checks at each corresponding planting date. The gross value added per acre when compared to the checks for each planting date were: \$6.45 per acre for the early planting, \$8.94 per acre for the mid planting, and \$23.67 per acre for the late planting (Table 11) (Figure 2). There were no other positive gross returns among the specialty groups at any planting date.

In the \$1.00 premium per bushel model, each planting of the rs2/rs3 type yielded positive gross returns when compared to the checks at each corresponding planting date. The gross value added per acre when compared to the checks for each planting date were: \$21.40 per acre for the early planting, \$23.44 for the mid planting, and \$35.72 for the late planting (Table 12) (Figure 3). There were no other positive gross returns among the specialty groups at any planting date.

The early planting date of the rs2/rs3 group yielded four bushels per acre less than the full season check, while the mid-planting yielded almost six bushels per acre less (Tables 10, 11, 12). Results from this study indicate that at the lowest estimation of a potential premium, the high sucrose/low RFO soybeans must yield within three bushels per acre to break even when compared to a commodity-type soybean crop. At the highest estimation of a potential premium, the specialty soybeans will break even when yielding around six bushels per acre less than the commodity-type soybeans. In order for high sucrose/low RFO soybeans to be economically feasible for soybean producers to grow instead of commodity type cultivars, the high sucrose/low RFO cultivar grown must be able to yield similarly to comparable commodity-type cultivars in order to improve profitability.

Another important trend observed in this experiment was that as planting date was delayed, the gross value per acre increased across all specialty groups. This can be explained by evaluating the yield data for each group across planting dates. With each subsequent planting date, the order remained the same throughout, but the magnitude of difference between the yields of each group were smaller with the later plantings. The resulting smaller yield differences between each group allowed for the premium of the specialty groups to have an increased opportunity to compensate for the yield drag that was generally seen among the specialty groups. Knowing this information from our experiment, it has become clear that these specialty soybeans would provide the best opportunity for improved profitability in a double-cropping production system in Missouri, especially the genotype XD3553, which consistently yielded among the top check cultivars in the experiment while having an exceptional carbohydrate profile.

Another potential option to increase profitability would be to stack this trait with another quality trait, such as high-oleic acid, which has produced average premiums of \$0.50 per bushel (Parcell et al., 2014); (United Soybean Board, 2016). Adding multiple quality traits such as these can improve value creation potential, and allows the opportunity for both the meal and oil components to yield premiums from processors. This research shows that the high sucrose/low RFO trait can improve profitability for producers. This trait also has the ability to be stacked with another quality trait to add further value to soybeans produced for specialty markets. More research is necessary to investigate the economic feasibility of stacking the high sucrose/low RFO trait with other quality traits such as high oleic acid.

## Tables

**Table 1.** Entry list of soybean genotypes with listed allelic mutation.

<b>Genotype</b>	<b>Type<sup>†</sup></b>	<b>MG</b>	<b>Developing Institution</b>
XD3553	rs2/rs3	3	Schillinger Genetics, Inc.
KB12-31#1027	rs2/rs3	3	Univ. of Missouri
e3011	rs2	3	Schillinger Genetics, Inc.
IA3051HS	rs2	3	Iowa St. Univ.
IA2104HS	rs2	3	Iowa St. Univ.
IA3023	Check	3	Iowa St. Univ.
IA3024	Check	3	Iowa St. Univ.
LD07-3395bf	Check	3	Univ. of Illinois
AG3231	Check	3	Monsanto Company
NK S39-U2	Check	3	Syngenta AG
R07-2000	MIPS1	4	Univ. of Arkansas
R07-2001	MIPS1	4	Univ. of Arkansas
KB13-7#1102	rs2/rs3	4	Univ. of Missouri
CR13-399	rs2	4	Univ. of Missouri
CR14-443	rs2	4	Univ. of Missouri
444D212P	Check	4	Schillinger Genetics, Inc.
Ellis	Check	4	UniSouth Genetics, Inc.
AG4232	Check	4	Monsanto Company
AG4632	Check	4	Monsanto Company
94Y23	Check	4	DuPont Pioneer

<sup>†</sup> Type = type of genotype based on allelic mutation (MIPS1, rs2, rs2/rs3, checks).

**Table 2.** Planting dates and monthly average of daily maximum air temperature (T max in °C), daily minimum air temperature (T min in °C), and total monthly rainfall (mm) for each growing season and environment in which the genotypes grown for this study were evaluated.

		2015			2016		
		Columbia	Novelty	Portageville	Columbia	Novelty	Portageville
<b>May</b>	T max	23.3	22.7	25.9	22.8	21.9	24.4
	T min	13.0	12.1	15.8	11.0	10.9	14.3
	Rainfall	140	119	126	81	108	94
<b>June</b>	T max	27.9	27.5	31.6	30.7	30.4	31.4
	T min	18.7	17.5	21.4	18.8	18.0	20.8
	Rainfall	130	322	91	29	38	50
<b>July</b>	T max	29.7	29.4	32.6	30.0	28.9	32.0
	T min	20.1	19.0	23.2	19.9	18.9	22.4
	Rainfall	204	257	70	274	116	90
<b>August</b>	T max	28.5	27.9	30.5	28.9	28.3	30.4
	T min	17.4	16.9	19.0	19.2	18.8	21.8
	Rainfall	106	106	50	149	206	137
<b>September</b>	T max	27.7	27.5	28.5	27.3	26.6	29.6
	T min	15.6	14.9	16.0	15.5	15.1	16.9
	Rainfall	21	35	19	143	45	29
<b>October</b>	T max	20.4	19.3	22.1	22.3	21.2	25.7
	T min	7.8	6.9	9.7	10.2	9.8	12.2
	Rainfall	25	52	73	25	52	30
<b>Planting Dates</b>	Early	May 6	—	May 13	May 5	May 24	May 16
	Mid	June 10	June 5	May 29	May 25	June 15	June 1
	Late	June 24	July 14	June 16	June 15	July 19	June 8

**Table 3.** Overall Analysis of variance (ANOVA) for yield and seed carbohydrates for twenty genotypes grown in 2015 and 2016; ten specialty lines bred for an improved carbohydrate profile, and ten check cultivars.

<b>Source of Effects</b>	<b>Suc</b>	<b>Raff</b>	<b>Stac</b>	<b>RFOs</b>	<b>Yield</b>
Environment (Env)	***	***	***	***	***
Planting Date (PD)	**	NS	NS	NS	***
Env x PD	NS	NS	NS	NS	***
Type (T)†	***	***	***	***	***
Env x T	***	***	***	***	***
PD x T	NS	*	*	NS	***
Env x PD x T	NS	NS	NS	NS	NS

† Type = type of genotype based on allelic mutation (MIPS1, rs2, rs2/rs3, checks).

§ Suc = sucrose concentration ( $\text{mg g}^{-1}$ ); Raff = raffinose concentration ( $\text{mg g}^{-1}$ ); Stac = stachyose concentration ( $\text{mg g}^{-1}$ ); RFOs = Raffinose Family of Oligosaccharides (raffinose + stachyose) ( $\text{mg g}^{-1}$ ); Yield = seed yield ( $\text{kg ha}^{-1}$ ).

NS not significant at the 0.05 probability level.

\* significant at the 0.05 probability level.

\*\* significant at the 0.01 probability level.

\*\*\* significant at the 0.001 probability level.

**Table 4.** Yield and seed carbohydrates of twenty genotypes as influenced by planting date (early, mid, late) averaged across environments, years, and maturity group. Included are: ten specialty lines bred for an improved carbohydrate profile, and ten check cultivars.

Type†	Suc‡	RFOs	Yield	Value Added to Base		
				\$0.50/bu. Premium	\$0.75/bu. Premium	\$1.00/bu. Premium
<b>Check</b>						
Early	47.0	52.2	4279.8	\$653.27	\$653.27	\$653.27
Mid	49.5	53.7	4132.3	\$630.75	\$630.75	\$630.75
Late	50.9	54.3	3322.5	\$507.15	\$507.15	\$507.15
<b>MIPS1</b>						
Early	90.5	11.7	2884.4	-\$190.06	-\$178.60	-\$167.13
Mid	91.7	13.8	2916.4	-\$162.41	-\$150.82	-\$139.22
Late	95.7	11.9	2835.2	-\$51.84	-\$40.57	-\$29.30
<b>rs2</b>						
Early	57.1	25.5	3058.0	-\$162.18	-\$150.03	-\$137.87
Mid	59.8	25.3	3014.2	-\$146.70	-\$134.72	-\$122.74
Late	62.4	24.2	2472.8	-\$110.04	-\$100.21	-\$90.38
<b>rs2/rs3</b>						
Early	65.7	22.4	4012.6	-\$8.89	\$7.06	\$23.01
Mid	67.1	21.5	3895.4	-\$5.19	\$10.29	\$25.78
Late	69.4	23.5	3238.1	\$12.86	\$25.73	\$38.60
<b>LSD§</b>	1.9	1.3	121.1			

† Type = type of genotype based on allelic mutation.

‡ Suc = sucrose concentration (mg g<sup>-1</sup>); RFOs = Raffinose Family of Oligosaccharides (raffinose + stachyose) (mg g<sup>-1</sup>); Yield = seed yield (kg ha<sup>-1</sup>); Value Added to Base = The gross value added per acre as compared to the checks at each corresponding planting date (\$/acre).

§ LSD = least significant difference at the 0.05 probability level.

**Table 5.** Yield and seed carbohydrates of eighteen genotypes as influenced by planting date (early, mid, late) at Columbia in 2015. Included are: nine specialty lines bred for an improved carbohydrate profile, and nine check cultivars.

2015 Columbia						
Type†	Suc‡	RFOs	Yield	Value Added to Base		
				\$0.50/bu. Premium	\$0.75/bu. Premium	\$1.00/bu. Premium
<b>Check</b>						
Early	42.4	44.9	4551.9	\$694.80	\$694.80	\$694.80
Mid	42.6	47.0	3771.7	\$575.71	\$575.71	\$575.71
Late	44.8	48.0	2918.4	\$445.46	\$445.46	\$445.46
<b>MIPS1</b>						
Early	68.5	10.8	1748.5	-\$414.01	-\$407.06	-\$400.11
Mid	76.3	12.4	1896.5	-\$271.15	-\$263.61	-\$256.08
Late	75.7	11.0	2135.2	-\$102.57	-\$94.09	-\$85.60
<b>rs2</b>						
Early	57.2	22.0	3013.3	-\$210.90	-\$198.92	-\$186.94
Mid	51.6	19.8	2995.6	-\$94.65	-\$82.74	-\$70.83
Late	54.4	19.6	2146.6	-\$100.74	-\$92.21	-\$83.68
<b>rs2/rs3</b>						
Early	53.1	20.1	4270.4	-\$9.02	\$7.96	\$24.93
Mid	62.5	22.0	4109.0	\$84.15	\$100.49	\$116.82
Late	61.3	21.7	2922.0	\$23.78	\$35.39	\$47.01
<b>LSD§</b>	3.8	2.3	300.0			

† Type = type of genotype based on allelic mutation.

‡ Suc = sucrose concentration ( $\text{mg g}^{-1}$ ); RFOs = Raffinose Family of Oligosaccharides (raffinose + stachyose) ( $\text{mg g}^{-1}$ ); Yield = seed yield ( $\text{kg ha}^{-1}$ ); Value Added to Base = The gross value added per acre as compared to the checks at each corresponding planting date (\$/acre).

§ LSD = least significant difference at the 0.05 probability level.

**Table 6.** Yield and seed carbohydrates of eighteen genotypes as influenced by planting date (early, mid, late) at Portageville in 2015. Included are: nine specialty lines bred for an improved carbohydrate profile, and nine check cultivars.

2015 Portageville						
Type†	Suc‡	RFOs	Yield	Value Added to Base		
				\$0.50/bu. Premium	\$0.75/bu. Premium	\$1.00/bu. Premium
<b>Check</b>						
Early	44.0	54.9	4484.4	\$684.50	\$684.50	\$684.50
Mid	46.6	57.1	4542.8	\$693.41	\$693.41	\$693.41
Late	48.0	56.4	4434.8	\$676.93	\$676.93	\$676.93
<b>MIPS1</b>						
Early	96.1	11.9	3852.1	-\$65.89	-\$50.58	-\$35.27
Mid	88.8	16.5	3710.8	-\$97.50	-\$82.75	-\$67.99
Late	95.0	12.4	3711.2	-\$80.95	-\$66.19	-\$51.44
<b>rs2</b>						
Early	47.2	27.0	3263.9	-\$160.35	-\$147.38	-\$134.40
Mid	54.7	27.8	3163.9	-\$185.32	-\$172.75	-\$160.17
Late	58.4	26.8	3084.5	-\$181.59	-\$169.33	-\$157.07
<b>rs2/rs3</b>						
Early	57.5	26.6	4249.8	-\$2.02	\$14.87	\$31.76
Mid	61.5	26.7	4256.1	-\$9.93	\$6.99	\$23.91
Late	62.6	29.3	4756.9	\$86.98	\$105.89	\$124.80
<b>LSD§</b>	3.2	2.4	293.2			

† Type = type of genotype based on allelic mutation.

‡ Suc = sucrose concentration ( $\text{mg g}^{-1}$ ); RFOs = Raffinose Family of Oligosaccharides (raffinose + stachyose) ( $\text{mg g}^{-1}$ ); Yield = seed yield ( $\text{kg ha}^{-1}$ ); Value Added to Base = The gross value added per acre as compared to the checks at each corresponding planting date (\$/acre).

§ LSD = least significant difference at the 0.05 probability level.

**Table 7.** Yield and seed carbohydrates of twenty genotypes as influenced by planting date (early, mid, late) at Columbia in 2016. Included are: ten specialty lines bred for an improved carbohydrate profile, and ten check cultivars.

2016 Columbia						
Type†	Suc‡	RFOs	Yield	Value Added to Base		
				\$0.50/bu. Premium	\$0.75/bu. Premium	\$1.00/bu. Premium
<b>Check</b>						
Early	52.2	53.7	4742.8	\$723.94	\$723.94	\$723.94
Mid	55.1	54.3	4791.1	\$731.31	\$731.31	\$731.31
Late	58.1	55.1	3497.7	\$533.89	\$533.89	\$533.89
<b>MIPS1</b>						
Early	111.7	9.4	3748.1	-\$122.03	-\$107.13	-\$92.24
Mid	107.2	11.9	3888.2	-\$106.91	-\$91.45	-\$76.00
Late	116.8	10.1	3378.0	\$8.58	\$22.01	\$35.44
<b>rs2</b>						
Early	60.8	25.4	3557.1	-\$152.71	-\$138.57	-\$124.43
Mid	60.7	24.9	3669.2	-\$142.08	-\$127.49	-\$112.91
Late	64.5	25.7	2447.8	-\$140.80	-\$131.07	-\$121.34
<b>rs2/rs3</b>						
Early	66.3	20.0	3768.7	-\$118.73	-\$103.74	-\$88.76
Mid	68.2	19.7	4044.0	-\$81.89	-\$65.81	-\$49.74
Late	67.8	19.4	2617.9	-\$113.48	-\$103.07	-\$92.67
<b>LSD§</b>	3.5	2.2	225.4			

† Type = type of genotype based on allelic mutation.

‡ Suc = sucrose concentration ( $\text{mg g}^{-1}$ ); RFOs = Raffinose Family of Oligosaccharides (raffinose + stachyose) ( $\text{mg g}^{-1}$ ); Yield = seed yield ( $\text{kg ha}^{-1}$ ); Value Added to Base = The gross value added per acre as compared to the checks at each corresponding planting date (\$/acre).

§ LSD = least significant difference at the 0.05 probability level.

**Table 8.** Yield and seed carbohydrates of twenty genotypes as influenced by planting date (early, mid, late) at Novelty in 2016. Included are: ten specialty lines bred for an improved carbohydrate profile, and ten check cultivars.

2016 Novelty						
Type†	Suc‡	RFOs	Yield	Value Added to Base		
				\$0.50/bu. Premium	\$0.75/bu. Premium	\$1.00/bu. Premium
<b>Check</b>						
Early	55.6	55.2	4390.6	\$670.18	\$670.18	\$670.18
Mid	59.4	55.3	4120.6	\$628.97	\$628.97	\$628.97
Late	62.4	53.4	2400.6	\$366.43	\$366.43	\$366.43
<b>MIPS1</b>						
Early	107.8	9.2	1784.0	-\$383.69	-\$376.60	-\$369.51
Mid	114.1	9.2	2014.7	-\$305.43	-\$297.42	-\$289.41
Late	112.2	10.0	1927.5	-\$56.89	-\$49.23	-\$41.57
<b>rs2</b>						
Early	69.0	24.8	3068.5	-\$177.41	-\$165.21	-\$153.02
Mid	69.4	23.0	2825.8	-\$175.17	-\$163.94	-\$152.71
Late	76.1	23.6	1988.1	-\$47.16	-\$39.26	-\$31.35
<b>rs2/rs3</b>						
Early	81.1	22.7	4159.1	-\$2.27	\$14.26	\$30.79
Mid	78.3	20.7	3834.4	-\$13.20	\$2.04	\$17.28
Late	84.6	23.2	2510.8	\$36.78	\$46.76	\$56.74
<b>LSD§</b>	4.0	2.6	321.8			

† Type = type of genotype based on allelic mutation.

‡ Suc = sucrose concentration ( $\text{mg g}^{-1}$ ); RFOs = Raffinose Family of Oligosaccharides (raffinose + stachyose) ( $\text{mg g}^{-1}$ ); Yield = seed yield ( $\text{kg ha}^{-1}$ ); Value Added to Base = The gross value added per acre as compared to the checks at each corresponding planting date (\$/acre).

§ LSD = least significant difference at the 0.05 probability level.

**Table 9.** Yield and seed carbohydrates of twenty genotypes as influenced by planting date (early, mid, late) at Portageville in 2016. Included are: ten specialty lines bred for an improved carbohydrate profile, and ten check cultivars.

2016 Portageville						
Type†	Suc‡	RFOs	Yield	Value Added to Base		
				\$0.50/bu. Premium	\$0.75/bu. Premium	\$1.00/bu. Premium
<b>Check</b>						
Early	38.9	50.5	3909.4	\$596.73	\$596.73	\$596.73
Mid	42.8	53.4	3429.4	\$523.46	\$523.46	\$523.46
Late	39.0	55.4	3377.4	\$515.53	\$515.53	\$515.53
<b>MIPS1</b>						
Early	71.9	15.7	3188.1	-\$84.75	-\$72.08	-\$59.41
Mid	76.5	14.4	3036.6	-\$35.82	-\$23.75	-\$11.68
Late	82.9	11.6	2988.3	-\$35.64	-\$23.76	-\$11.88
<b>rs2</b>						
Early	51.3	28.1	2401.0	-\$211.15	-\$201.61	-\$192.07
Mid	56.6	28.2	2383.3	-\$140.73	-\$131.26	-\$121.78
Late	56.5	25.1	2704.6	-\$81.19	-\$70.44	-\$59.69
<b>rs2/rs3</b>						
Early	70.8	22.9	3662.7	-\$8.54	\$6.02	\$20.58
Mid	67.6	20.8	3391.3	\$21.15	\$34.63	\$48.11
Late	71.1	23.9	3393.0	\$29.36	\$42.84	\$56.33
<b>LSD§</b>	5.4	3.7	228.1			

† Type = type of genotype based on allelic mutation.

‡ Suc = sucrose concentration ( $\text{mg g}^{-1}$ ); RFOs = Raffinose Family of Oligosaccharides (raffinose + stachyose) ( $\text{mg g}^{-1}$ ); Yield = seed yield ( $\text{kg ha}^{-1}$ ); Value Added to Base = The gross value added per acre as compared to the checks at each corresponding planting date (\$/acre).

§ LSD = least significant difference at the 0.05 probability level.

**Table 10.** Gross added value per acre of specialty soybean genotypes compared to the checks at each corresponding planting date at a \$0.50/bushel premium on top of current market value\* for soybeans with high sucrose and low RFOs across three planting dates.

	Check – early	Check – mid	Check – late	MIPS - early	MIPS - mid	MIPS - late
<b>Yield (bu./ac.)</b>	63.8	61.6	49.5	43	43.5	42.2
<b>Premium (\$/bu.)</b>				\$0.50	\$0.50	\$0.50
<b>Total value per acre (\$/ac.)</b>	\$612.48	\$591.36	\$475.20	\$434.30	\$439.35	\$426.22
<b>Value Added to Base</b>		-\$21.12	-\$137.28	-\$178.18	-\$152.01	-\$48.98

	rs2 - early	rs2 - mid	rs2 - late	rs2/rs3 - early	rs2/rs3 - mid	rs2/rs3 - late
<b>Yield (bu./ac.)</b>	45.6	44.9	36.8	59.8	58.0	48.2
<b>Premium (\$/bu.)</b>	\$0.50	\$0.50	\$0.50	\$0.50	\$0.50	\$0.50
<b>Total value per acre (\$/ac.)</b>	\$460.56	\$453.49	\$371.68	\$603.98	\$585.80	\$486.82
<b>Value Added to Base</b>	-\$151.92	-\$137.87	-\$103.52	-\$8.50	-\$5.56	\$11.62

\* Current market value as of 3/31/17 (\$9.60 per bushel) was used for this model

**Table 11.** Gross added value per acre of specialty soybean genotypes compared to the checks at each corresponding planting date at a \$0.75/bushel premium on top of current market value\* for soybeans with high sucrose and low RFOs across three planting dates.

	Check - early	Check - mid	Check - late	MIPS - early	MIPS - mid	MIPS - late
<b>Yield (bu./ac.)</b>	63.8	61.6	49.5	43	43.5	42.2
<b>Premium (\$/bu.)</b>				\$0.75	\$0.75	\$0.75
<b>Total value per acre (\$/ac.)</b>	\$612.48	\$591.36	\$475.20	\$445.05	\$450.23	\$436.77
<b>Value Added to Base</b>		-\$21.12	-\$137.28	-\$167.43	-\$141.14	-\$38.43

	rs2 - early	rs2 - mid	rs2 - late	rs2/rs3 - early	rs2/rs3 - mid	rs2/rs3 - late
<b>Yield (bu./ac.)</b>	45.6	44.9	36.8	59.8	58.0	48.2
<b>Premium (\$/bu.)</b>	\$0.75	\$0.75	\$0.75	\$0.75	\$0.75	\$0.75
<b>Total value per acre (\$/ac.)</b>	\$471.96	\$464.72	\$380.88	\$618.93	\$600.30	\$498.87
<b>Value Added to Base</b>	-\$140.52	-\$126.65	-\$94.32	\$6.45	\$8.94	\$23.67

\* Current market value as of 3/31/17 (\$9.60 per bushel) was used for this model

**Table 12.** Gross added value per acre of specialty soybean genotypes compared to the checks at each corresponding planting date at a \$1.00/bushel premium on top of current market value\* for soybeans with high sucrose and low RFOs across three planting dates.

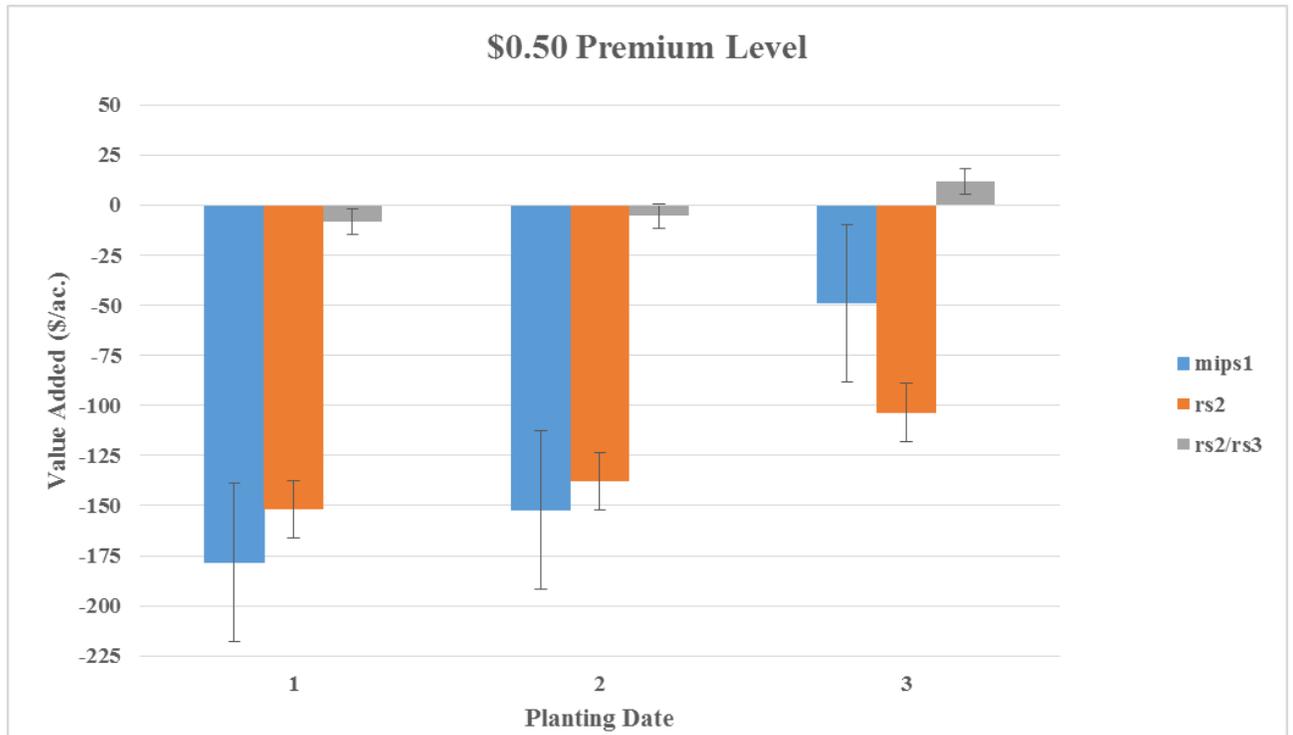
	Check - early	Check - mid	Check - late	MIPS - early	MIPS - mid	MIPS - late
<b>Yield (bu./ac.)</b>	63.8	61.6	49.5	43	43.5	42.2
<b>Premium (\$/bu.)</b>				\$1.00	\$1.00	\$1.00
<b>Total value per acre (\$/ac.)</b>	\$612.48	\$591.36	\$475.20	\$455.80	\$461.10	\$447.32
<b>Value Added to Base</b>		-\$21.12	-\$137.28	-\$156.68	- \$130.26	-\$27.88

	rs2 - early	rs2 - mid	rs2 - late	rs2/rs3 - early	rs2/rs3 - mid	rs2/rs3 - late
<b>Yield (bu./ac.)</b>	45.6	44.9	36.8	59.8	58.0	48.2
<b>Premium (\$/bu.)</b>	\$1.00	\$1.00	\$1.00	\$1.00	\$1.00	\$1.00
<b>Total value per acre (\$/ac.)</b>	\$483.36	\$475.94	\$390.08	\$633.88	\$614.80	\$510.92
<b>Value Added to Base</b>	-\$129.12	-\$115.42	-\$85.12	\$21.40	\$23.44	\$35.72

\* Current market value as of 3/31/17 (\$9.60 per bushel) was used for this model

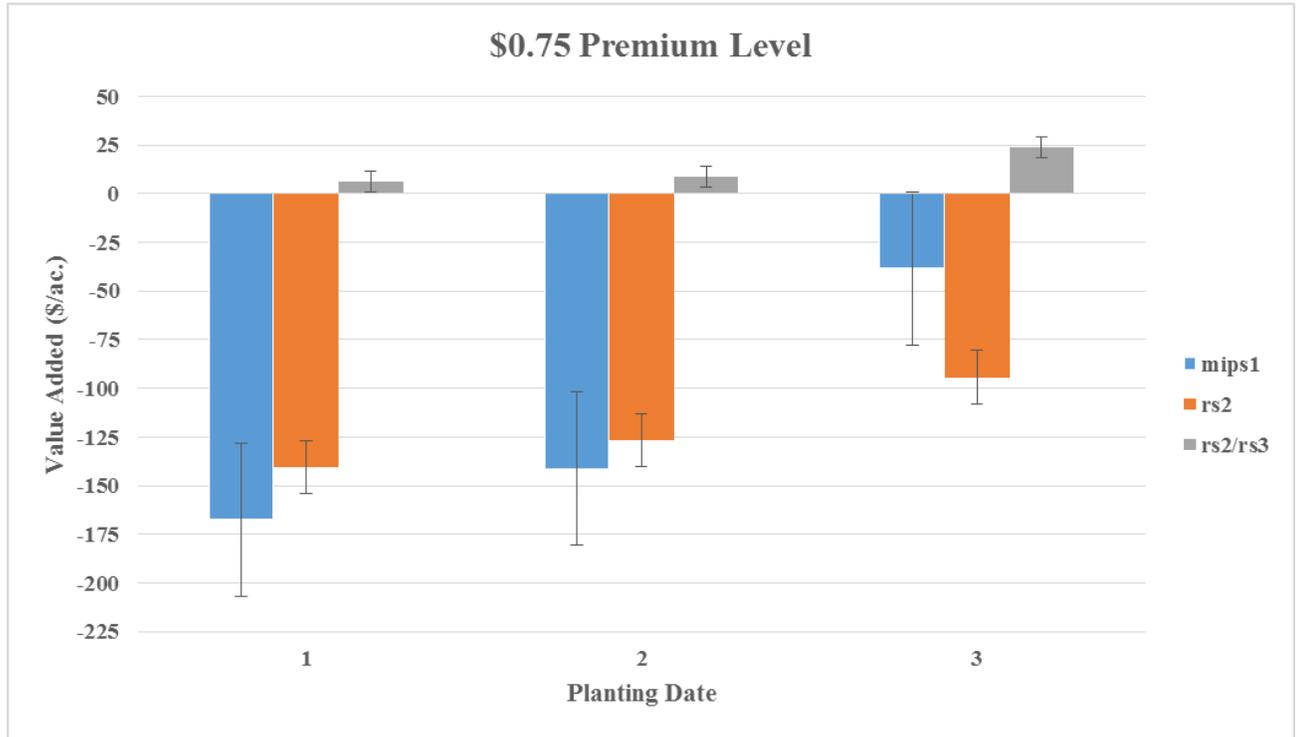
## Figures

**Figure 1.** Gross added value per acre of specialty soybean genotypes compared to the checks at each corresponding planting date at a \$0.50/acre premium on top of current market value\* for soybeans with high sucrose and low RFOs across three planting dates.



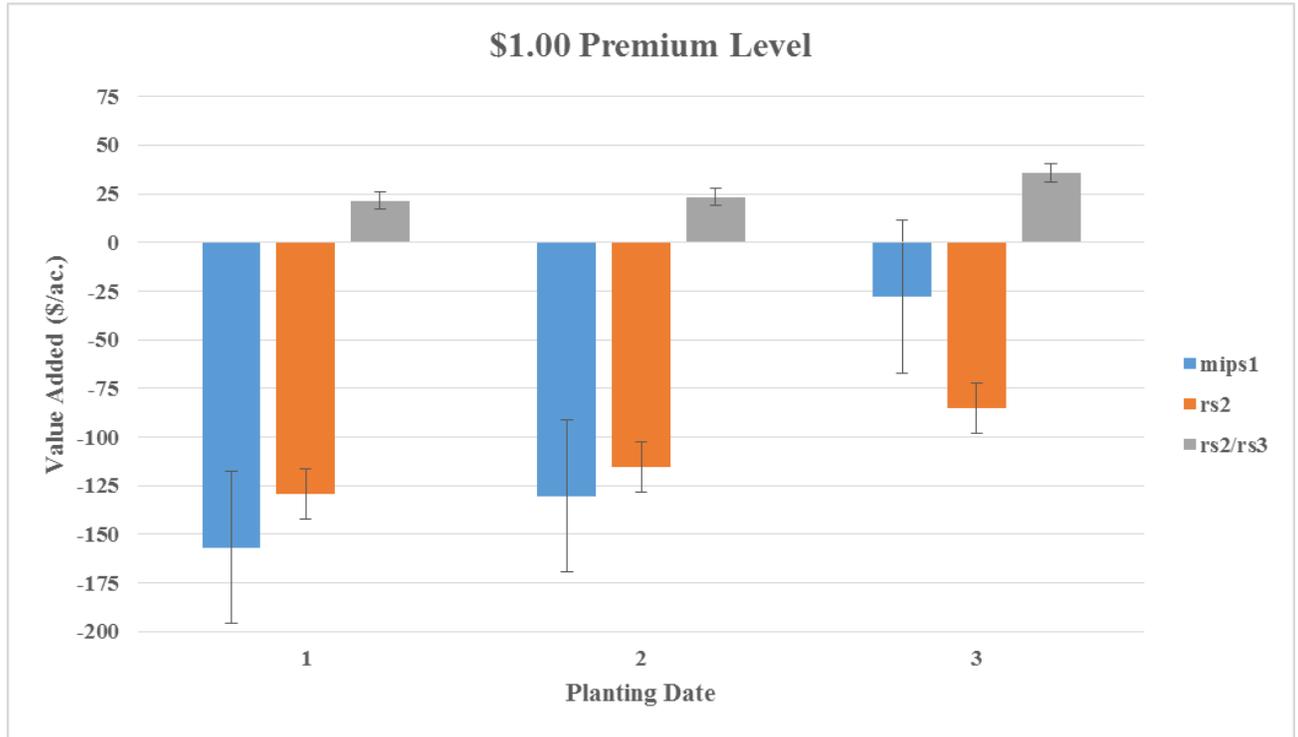
\* Current market value as of 3/31/17 (\$9.60 per bushel) was used for this model

**Figure 2.** Gross added value per acre of specialty soybean genotypes compared to the checks at each corresponding planting date at a \$0.75/acre premium on top of current market value\* for soybeans with high sucrose and low RFOs across three planting dates.



\* Current market value as of 3/31/17 (\$9.60 per bushel) was used for this model

**Figure 3.** Gross added value per acre of specialty soybean genotypes compared to the checks at each corresponding planting date at a \$1.00/acre premium on top of current market value\* for soybeans with high sucrose and low RFOs across three planting dates.



\* Current market value as of 3/31/17 (\$9.60 per bushel) was used for this model

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