112

Improving the Food Quality of Sorghum

Utilizing functional genomics to identify novel starch phenotypes in EMS sorghum mutants

Cross, Valerie; McKneight, Molly; Tuinstra, Mitchell
Agronomy Dept., Purdue University, Lilly Hall of Life Sciences, 915 W. State Street, West Lafayette, IN 47907

Abstract

Sorghum bicolor is the main food source for 500 million people around the world. Sorghum is a highly versatile food source that is widely utilized in marginal environments. The food quality of sorghum, however, is relatively poor, and research and innovation in the area of sorghum is severely lacking. This research focused on identifying mutants with the potential to produce a higher food quality through modification of the starch properties. Eleven different lines of mutant sorghum were analyzed to determine if any of the lines display characteristics that deviate from the control sorghum line, Tx623. Three different characteristics of the starch, amylose content, retrogradation profiles, and gelatinization profiles, were analyzed. Two different lines displayed significantly higher levels of amylose than Tx623. Both of these lines had mutations in the 1,4-alpha-glucan-branching enzyme. These two lines also displayed increased retrogradation and decreased enthalpy of gelatinization and temperature of gelatinization. These novel starch phenotypes of sorghum could improve the utilization of the grain in food and beverage applications.

Background

Sorghum is the fifth most grown cereal crop in the world[6]. It is able to grow in marginal soils, survive droughts, and enter a dormant state under stress. Sorghum, therefore, is a highly versatile food source that is utilized in marginal environments in developing countries as the main source of starch. Sorghum flour can be cooked into semi-leavened breads, couscous, dumplings, porridges, and beer. Unfortunately, sorghum has been generally disregarded by modern improvements in agriculture.

The major component of sorghum flour is starch. Starch is composed of two homoploymers, amylose, a linear molecule, and amylopectin, a highly branched molecule[5]. Flour has different properties depending on the percentages of these two molecules within the starch. The amylose content was of interest in this study due to its influence over gelatinization, which is the process of breaking down the bonds within starch molecules which irreversibly dissolves the starch granule, and retrogradation, which is when the amylose and amylopectin chains rearrange themselves in a crystalline structure[1]. Both gelatinization and retrogradation are key components of dough and porridge quality. Mutations in 11 created lines targeted enzymes involved in the creating amylose or amylopectin. These lines, and their corresponding information are displayed below.

EMS#	starch metabolism target
0580-2	starch branching enzyme 2.2
0878-1	starch branching enzyme 2.2
1297-1	ADP-glucose pyrophosphorylase
1930-2	isoamylase 3

EMS#	starch metabolism target
2089-1	starch synthase 4
2247-1	ZPU1 (pullulanase)
2617-1	isoamylase 1
3218-1	1,4-alpha-glucan-branching enzyme

EMS#	starch metabolism target
3568-2	1,4-alpha-glucan-branching enzyme
3711-1	starch synthase 2
4015-1	ZPU1 (pullulanase)

Materials and Methods

Isolating Starch from Sorghum Flour[3]

All steps were performed eight samples at a time for a total of three times.

2.5g flour and 50mL of 0.125M sodium borate buffer in a beaker → Sonicate 100 seconds at 60 Hz in ice water bath and transfer to centrifuge tubes → Centrifuge 10 minutes at 3200 g and decant liquid → Wash pellet through a 62µm screen with distilled water and plastic spatula → Re-suspend pellet in 40mL distilled water → Centrifuge 5 minutes at 3200 g and rinse through the screen → Dry starch pellet via lyophilization and crushed with glass rod

Repeat 3 times

Determining Amylose Content[2]

All steps were performed eight samples at a time for a total of three times

Add 5 mg of each sample and 1 ml of 90% DMSO to each 2 mL tubes → Heat to 95°C in heating block for 60 minutes; vortex every 10 minutes → Cool 5 minutes and transfer 100µL of each to plate in order starting in the second row → Prepare the standard curve in the top row leaving two blank wells on either end → Add 100µL of 90% DMSO with 3.04 g/L of iodine to each well and shake the plate for 5 minutes → Prepare the blanks → Transfer 20µL from each cell to a new spectrophotometer reading plate → Add 180µL of deionized water to each cell, agitate, and read the absorbance at 620nm and 510nm

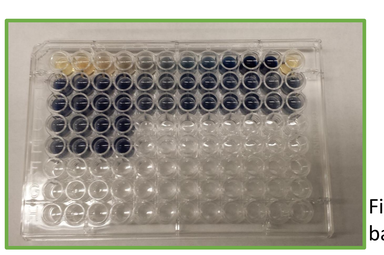


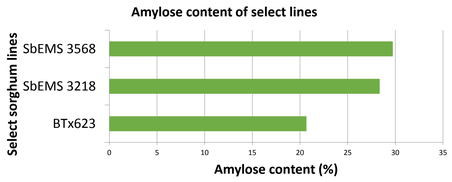
Figure 1: Iodine binds to the amylose to produce different intensity blue coloration based on the amount of amylose in the sample.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) analysis was performed by the Whistler Center for Carbohydrate Research (West Lafayette, IN)

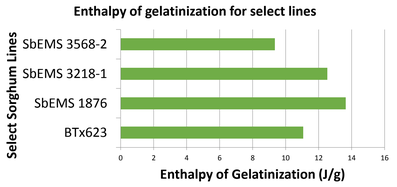
Results

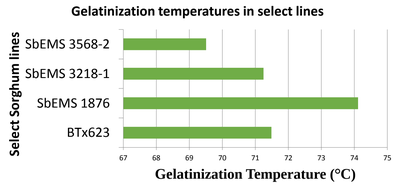
From the 96-well plate iodine binding assay, a standard curve is generated with an equation of a line based on different percentages of amylose. This equation is used to calculate the amylose percentage in the samples. This research identified two mutant lines that displayed different phenotypes from the control, BTx623.

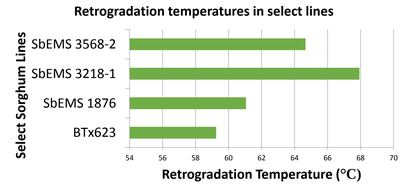


Line SbEMS 3218 displayed a 7.7% increase in amylose content over Tx623. Line SbEMS 3568 displayed a 9.06% increase in amylose content over BTx623.

The DSC results are shown below in bar graph form comparing the two lines of interest to BTx623. Data on a waxy line, SbEMS1876, of sorghum was included because waxy lines have virtually no amylose in the starch.







Line SbEMS 3218 displayed a very slight (0.24°C) decrease in gelatinization temperature, an 8.68°C increase in retrogradation temperature, and a very slight increase in gelatinization enthalpy. Line SbEMS 3568 displayed a 1.98°C decrease in gelatinization temperature, a 5.41°C increase in retrogradation temperature, and a 1.71 J/g decrease in gelatinization enthalpy.

The mutant lines were also genetically sequenced. Line SbEMS 3218 contains a point mutation that caused a stop codon in the middle of the gene that coded for 1,4-alpha-glucan-branching enzyme. Line SbEMS 3568 contains a point mutation that caused a stop codon to occur in the very first codon of the gene that codes for 1,4-alpha-glucan-branching enzyme.

Conclusions

The amount of amylose in the flour determines the quality of the dough. High amylose content generally results in changes in gelatinization and retrogradation temperatures [1]. The two lines identified as “increased amylose” lines displayed these changes. Lower gelatinization temperatures increase the viscoelasticity of the dough and increase the digestibility of the starches [1]. High amounts of retrogradation also equate to slower enzymatic digestion and a slower release of glucose into the blood stream[4]. This could make a person feel fuller longer and stretch the nutritional release of a meal further. Lower gelatinization enthalpy equates to less energy needed to gelatinize the starches, less heat, less time, and more convenient foods. The two mutant lines identified in this study each have two of these three desirable properties.

It is very likely that the mutations in the starch branching enzyme, 1,4-alpha-glucan-branching enzyme, are the cause of the changes in starch phenotype that were observed. The difference between the levels of amylose between the two lines with a mutation in the same gene is most likely due to the location of the mutation. Line SbEMS 3218 has a mutation in the middle of the gene so it possibly retains some function. Line SbEMS 3568 has a mutation in the very first codon that stops the entire gene from being translated. The challenge now is to integrate these mutations into elite sorghum germplasm and study how these mutations can positively affect applications in brewing and baking.

Further tests should be run on the future generations of these mutant, elite plants. Three further tests that could be beneficial to run on these samples are fluorophore-assisted carbohydrate electrophoresis (FACE), laser diffraction size particle analysis, and measurement of the resistant starches. The first test, FACE, measures the amylopectin branch chain length. The second test, laser diffraction size particle analysis allows us to determine the starch particle size distribution. The final test measures resistant starches. Previous research done in this lab determined that amylose content could also have an impact on how much resistant starch there is in the flour. All three of these tests would reveal further pheontypes of interest in improving the food quality of sorghum.

References

[1]Abah, J., Asemave, K., Shambe, T., Ubwa, S. (2012). Studies on the Gelatinization Temperature of Some Cereal Starches. ***International Journal of Chemistry, North America***.

[2]Bean, S.R., Herald, T.J., Kaufman, R.C., Shi, Y.C., Wilson, J.D. (2015) Development of a 96-well plate iodine binding assay for amylose content determination. *Carbohydrate Polymers 115, 444-447*.

[3]Bean, S.R., Park, S.H., Schober, T.J., and Wilson, J.D. (2006) Rapid Isolation of Sorghum and Other Cereal Starches Using Sonication. *Cereal Chemistry, 83(6), 611-616).*

[4]Copeland, L., Li, C., Niu, Q., Wang, S., and Wang, S. (2015) Starch Retrogradation: A Comprehensive Review. *Comprehensive Reviews in Food Science and Food Safety 14(5), 568-585.*

[5]Denyer, K., James, M.J., Myers, A.M. (2003). Starch synthesis in the cereal endosperm. *Current Opinion in Plant Biology, 6, 215-222.*

[6]USDA Natural Resources Conservation Service. (2014). *Sorghum bicolor.* http://plants.usda.gov/core/profile?symbol=sobi2