DEVELOPMENT OF HIGH-AMYLOSE CORN VARIETIES FOR NORTHEAST MISSOURI: A POSSIBLE SPECIALTY CROP OPPORTUNITY FOR LOCAL PRODUCERS.

Mark Campbell
Associate Professor, Truman State University

Jessica Ponder
Undergraduate Research Assistant

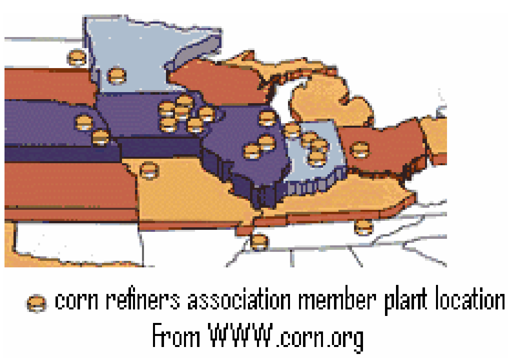
Courtney Bonney
Undergraduate Research Assistant

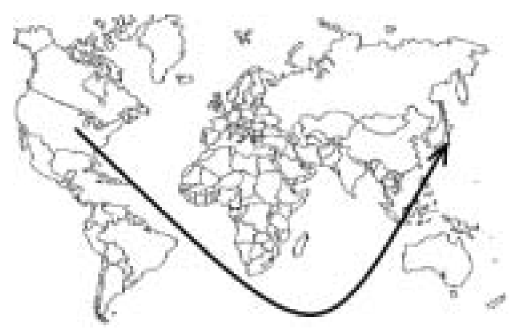
Overview:

Starch is the major component of cereal grains. In corn it makes up approximately 75% of its dry matter. Starch is a polymer with the simple sugar glucose serving as the repeating unit. Normally starch occurs in two forms: amylose, a linear molecule normally making up 25% of the starch, and amylopectin, a branched form comprising the remainder. For Missouri livestock growers, producers of ethanol and other end-users this composition of starch in our modern hybrids serves well and little or no modification is necessary. The purpose of our study is to develop a 'naturally-modified' specialty starch corn hybrid adapted to Northeast Missouri, and explore potential niche markets for its domestic use or for export.

Purified corn starch has countless industrial applications. Modification, either chemical or genetic, of the starch can provide added benefits to specific end-users. Starches are typically produced by wet milling operations, which consume approximately 2.6% of the nations total corn crop. Although none exist in Northeast Missouri, several are located in Southeast Iowa. In addition, corn is exported for overseas wet milling especially in industrial countries such as Japan; Northeast Missouri located along an established transport network is well situated for export. Both of these potential markets might benefit if a high-quality specialty starch hybrid required by wet mills were produced here (Figure 1).

Figure 1. Potential Markets for High-Amylose corn produced in Northern Missouri





High-amylose corn is one of several specialty starch hybrids of interest to wet mills. It is a non-GMO-type corn that utilizes traditional plant breeding techniques to increase the relative amount of amylose in the starch. Its generally grown under contract with a wet-mill and commands a substantial premium (Table 1), however, at least part of this is needed to offset the fact that it yields only 75-80% as much as normal hybrids, with lower test weights as well (Kansas State University, 1999). Missouri farmers may be aware of another specialty starch corn currently being grown in the state with 100% amylopectin. Known as ‘waxy’ corn it is produced by many Western Missouri corn growers under contract with the National Starch and Chemical Company in the Kansas City region. Waxy corn premiums, however, are not nearly as large as high-amylose corn, since they produce a normal yield.

Table 1. Market trends and premiums of some selected specialty corn types

	U.S. Acreage (1,000)				Price Premium Range
	1996	**1997**	**1998**	**1999**	
Waxy	400	420	500	525	$0.25 - 0.30
High Oil	400	700	900	1250	$0.25 - 0.30
High Amylose	35	35	35	45	$1.20 +

Source: U.S. Grains Council

As mentioned, normal corn generally has an amylose content of ~25% of total starch. High-amylose corn, on the other hand, includes all corn with an amylose content >50%. There are, however, a number of specific high-amylose classes including amylomaize V (>50% amylose) and amylomaize VII (>70% amylose). In the U.S., high-amylose corn varieties have been grown and produced over the past 30 years, mainly in the Eastern corn belt by two companies: National Starch and Chemical Co. and Cargill. Varieties are highly protected and until recently, there has been no public germplasm source available to breeders. High-amylose starches have been used traditionally as an ingredient in gum candies, adhesives for corrugated cardboard and as ‘sizing’ to strengthen yarn. For these applications end-users generally consider varieties with the highest amylose contents to be the most effective. Two recent advances that have increased interest in the use of high-amylose starches and thus have led to increases in its production. First, the development of thermoplastic starch (TPS) in biodegradable plastics has been found to produce thermoplastic materials such as shopping bags, bread bags, bait bags, over wrap, 'flushable' sanitary product ,packing material, and mulch film. Secondly, many national and international food companies are looking towards amylomaize as a source of resistant starch (RS), a type of starch that resist digestion. As a food additive consumers could benefit from RS added to processed food because it lowers the glycemic index. Additionally, RS behaves as dietary fiber and is believed to lower the risk of colon cancer when incorporated into the diet (Sajilata et al 2006).

Objective 1: Identify corn line with desired high-amylose genes:

Initial breeding studies at Truman State University have resulted in the development of a corn line possessing the desired high-amylose starch trait and has been designated as GEMS-0067. It possesses modifier genes that, together with the recessive amylose-extender (*ae*) allele, elevates starch amylose content to at least 70%. To our knowledge GEM-0067 represents the only public source of Amylomaize VII to date. Selection for high-amylose modifiers was made following the screening of many exotic varieites of corn maintianed the the National Plant Germplasm System (NPGS) and the Germplasm Enhancement of Maize (GEM) project both administered within the USDA-ARS. Our results revealed that two plant introductions (NRC 5357 Zia Pueblo, PI 218139 and Cochiti Pueblo NRC 5298, PI 218150) and one GEM breeding cross (GUAT209:S13) contributed high-amylose modifiers raising amylose levels to at least 70% in the F3 kernels. Only lines from GUAT209:S13 x (H99ae x OH43ae) were found to survive inbreeding. The others, although used for yield analyisis as described in the next section, were eventually dropped.

GUAT209:S13 is a 50% tropical exotic derived from crossing the accession Guatemala 209 (PI 498583) to a proprietary stiff stalk inbred from a private GEM cooperator. Evaluations made in Kirksville from 2002 to 2005 indicate that GEMS-0067 is a vigorous line with days to pollen shed occurring approximately four days before B73, with plant heights averaging 174 cm, exhibiting an upright leaf structure, with yellow kernels and red cob. Since the *ae* donor was from the non-stiff stalk source H99ae x OH43ae, GEMS-0067 is of mixed heterotic classification composed of 50% non-stiff stalk, and 50% stiff stalk. Since selections for the high-amylose trait were initially made solely on the colorimetric iodine affinity (IA) method which measures only "apparent amylose", starch was further characterized from GEMS-0067 and compared to several public inbreds, possessing *ae* but without high-amylose modifier genes, using additional analytical techniques (Table 2). Starch was isolated from two bulk samples (Sample 1 and 2) obtained from independent single-row plots derived from two S4 ears produced in 2004 of GEMS-0067. In addition, bulk samples from the converted lines H99ae, OH43ae, B89ae and B84ae (Samples 3,4,5 and 6 respectively) grown in the same year were used for starch isolation. Analysis of amylose using the IA method confirms the presence of modifiers in two samples obtained from GEMS-0067. Since apparent amylose can be elevated as the results of long chained amylopectins with the IA method, amylose was determined by using gel permeation chromatography (GPC) that physically separates and measures the amylose and amylopectin fractions. Although the GPC method generally gives higher amylose values of all samples compared to the IA method, GEMS-0067 displays a relatively higher amylose value than others, based on analysis of GPC amylose peaks. The resistant starch (RS) was also measured by using an AOAC method for total dietary fiber content for all samples. The RS contents of GEMS-0067 starches were approximately two times higher than that of the converted public lines, 39.4% and 11.5-19.1%, respectively. The RS content of the starch sample was positively correlated with the amylose content of the starch with a correlation coefficient (r) of 0.89. Thermal properties of the native high-amylose maize starch:water mixtures (3:1 ratio) as determined by Differential Scanning Calorimetery (DSC) were also determined. All the starch samples displayed similar onset gelatinization temperature (63.8-65.0^{o}C). Broader gelatinization temperature ranges, however, were observed for GEMS-0067 starches. The conclusion temperatures of these also varied from 104.8 to 106.7^{o}C, which were substantially higher in GEMS-0067 starch than that of the other starch samples (92.8 –94.2^{o}C). The results indicated that starch granules of GEMS-0067 samples were not completely gelatinized after cooking at boiling temperature likely a result of the increased amylose content.

Table 2. Confirmation of high-amylose trait in our line GEMS-0067

		RS†	% Amylose		Thermal properties measured by DSC‡			
Sample	Pedigree	%	IA§	GPC#	T_o	T_p	T_c	ΔH
1	GUAT209:S13 x (OH43aexH99ae) 4-4-2-1-1	43.2	69.8	89.3	65.0	92.1	106.7	6.2
2	GUAT209:S13 x (OH43aexH99ae) 4-4-2-1-2	39.4	72.0	86.1	64.2	85.2	104.8	7.1
3	H99ae	19.1	63.1	66.5	64.4	77.7	94.2	12.6
4	OH43ae	14.0	57.8	74.6	64.8	77.0	94.0	15.6
5	B89ae	14.9	56.7	67.0	64.3	80.5	92.8	14.2
6	B84ae	11.5	58.9	67.7	65.0	77.1	94.0	13.3
	mean	23.6	63.0	75.2	64.6	81.6	97.8	11.5
	s.e.	0.72	2.5	10.7	0.53	2.86	1.77	1.33

† Resistant starch
§ Amylose determined using iodine-affinity methods described by Williams (1959)
# Amylose determined by Gel permeation chromatography
‡ Differential Scanning Calorimetry of samples (~3.0 mg,dwb) and deionized water (~9.0 mg) were used for the analysis; T_0, T_p, T_c and ÷H are onset, peak, conclusion temperature, and enthalpy change, respectively.

Objective 2: Amylomaize VII single-cross hybrid evaluation using GEMS-0067 derived lines onto a private line:

In order to obtain some preliminary yield data from early generation amylomaize VII-type corn lines derived from GEMS-0067, a yield trial was conducted over two years (2004 and 2005) in Novelty, Missouri and Ames, Iowa. Agronomic data including yield are shown in Table 3. A number of F3 lines derived from crosses between the high-amylose modifier source Guat209//H99ae/Oh43ae, Zia Peublo//H99ae/Oh43ae, and Cochiti Peublo//H99ae/Oh43ae onto several released GEM lines previously selected for high yield, were crossed onto an OH43-type amylomaize VII tester and a Stiff-stalk-type amylomaize VII tester from a private company. In addition, a proprietary amylomaize VII check hybrid was included for comparison. In general, many of the GEM containing hybrids produced a greater yield than the proprietary check. Amylose data in Table 3 represents hybrid grain samples analyzed from only the 2004 growing season that was collected from selfed hybrid plants. Although many amylose values are below 70%, it is likely that continued inbreeding and selection could be made to fix the genes since they probably carry many of the modifying genes in these genetically variable, early lines. Of most immediate interest is the hybrid CHIS775:N1912-519-1-B-B///GUAT209:S13//OH43ae/H99ae //// ss tester yielding 100.0 bu/A with a 2004 amylose analysis of 69.3%.

Table 3. Pooled yield and agronomic characteristics of GEM x Proprietary tester hybrids grown in 2004 and 2005 in Ames, IA and Novelty, MO

SOURCE	Pedigree	WT	Yield(bu/ac)	MOIST	YM	twt	STAND	%stand	%stklg	%rtlg	% amylose 2004 *
03:403	DKXL370:N11a20-31-1-B-B-SIB/VGUAT209:S13/VOH43ae/H99ae /// OH43 tester	10.8	**86.2**	17.6	5.5	52.4	51.4	81.3	4.5	1.7	62.4
03:404	DKXL370:N11a20-31-1-B-B-SIB/VGUAT209:S13/VOH43ae/H99ae /// OH43 tester	10.9	**86.7**	18.2	5.3	51.7	57.6	91.1	4.4	1.4	71.8
03:405	DKXL370:N11a20-31-1-B-B-SIB/VGUAT209:S13/VOH43ae/H99ae /// OH43 tester	11.4	**90.4**	18.7	5.3	53.6	54.7	86.6	3.5	0.5	56.9
03:406	DKXL370:N11a20-31-1-B-B-SIB/VGUAT209:S13/VOH43ae/H99ae /// OH43 tester	11.6	**91.6**	19.5	5.3	52.5	57.0	90.2	6.2	1.2	60.6
03:415	AR16035:S02-615-1-B-B/VGUAT209:S13/VOH43ae/H99ae /// OH43 tester	8.9	**71.6**	18.0	4.4	52.8	50.5	80.1	3.9	0.5	65.6
03:416	AR16035:S02-615-1-B-B/VGUAT209:S13/VOH43ae/H99ae /// OH43 tester	10.2	**81.8**	17.9	3.1	50.1	47.6	75.2	4.1	1.8	56.3
03:417	AR16035:S02-615-1-B-B/VGUAT209:S13/VOH43ae/H99ae /// OH43 tester	11.7	**99.0**	15.6	6.3		50.5	78.9	2.9	1.8	53.9
03:418	AR16035:S02-615-1-B-B/VGUAT209:S13/VOH43ae/H99ae /// OH43 tester	11.0	**89.6**	17.3	5.9	52.6	50.4	79.9	6.1	1.9	61.7
03:407	Cuba164:S15-192-1-B-B/VNRC 5357 Zia Pueblo/VOH43ae/H99ae /// OH43 tester	8.9	**75.8**	15.9	4.8	49.5	51.3	81.2	6.9	0.6	64.2
03:413	Cuba164:S2008a-469-1-B-B/VNRC 5357 Zia Pueblo/VOH43ae/H99ae /// OH43 tester	11.1	**89.4**	16.3	6.3	51.5	47.1	74.8	5.0	1.2	61.6
03:421	AR01150:N04-213-1-B-B/VNRC 5357 Zia Pueblo/VOH43ae/H99ae /// OH43 tester	7.9	**65.1**	14.9	4.8	52.7	47.0	74.2	4.1	0.5	67.8
03:411	Cuba164:S15-253-1-B-B/VNRC 5357 Zia Pueblo/VOH43ae/H99ae /// OH43 tester	10.1	**85.0**	16.3	5.3		46.0	71.9	2.1	4.8	59.6
	Ave. of SS-type tester	**10.4**	**84.3**	**17.3**	**5.4**	**51.9**	**51.4**	**81.2**	**4.7**	**1.1**	
03:404	DKXL370:N11a20-31-1-B-B-SIB/VGUAT209:S13/VOH43ae/H99ae /// ss tester	12.2	**93.7**	20.6	4.7	51.1	56.4	89.3	5.6	2.7	61.3
03:405	DKXL370:N11a20-31-1-B-B-SIB/VGUAT209:S13/VOH43ae/H99ae /// ss tester	12.7	**99.2**	20.4	3.2	51.9	54.9	87.8	6.7	1.3	62.3
03:406	DKXL370:N11a20-31-1-B-B-SIB/VGUAT209:S13/VOH43ae/H99ae /// ss tester	12.5	**97.0**	20.6	3.2	52.7	54.5	86.2	7.3	1.8	64.7
03:414	CHIS775:N1912-519-1-B-B/VGUAT209:S13/VOH43ae/H99ae /// ss tester	12.3	**100.0**	17.3	6.3	52.0	51.8	81.6	9.1	1.6	69.3
03:415	AR16035:S02-615-1-B-B/VGUAT209:S13/VOH43ae/H99ae /// ss tester	11.9	**91.9**	21.2	4.8	51.5	55.4	87.7	8.4	0.7	70.3
03:416	AR16035:S02-615-1-B-B/VGUAT209:S13/VOH43ae/H99ae /// ss tester	13.3	**104.0**	19.1	5.9	51.8	54.2	85.8	5.6	0.7	65.6
03:417	AR16035:S02-615-1-B-B/VGUAT209:S13/VOH43ae/H99ae /// ss tester	12.6	**98.5**	18.2	5.7	54.2	54.0	85.5	5.9	0.2	57.3
03:418	AR16035:S02-615-1-B-B/VGUAT209:S13/VOH43ae/H99ae /// ss tester	11.1	**89.2**	18.5	5.5	53.8	52.6	83.2	4.6	2.9	60.8
03:420	AR01150:N04-046-1-B-B/VGUAT209:S13/VOH43ae/H99ae /// ss tester	13.8	**105.7**	22.3	3.3	50.9	60.2	95.3	10.3	1.1	23.6
03:401	DKXL370:N11a20-31-1-B-B-SIB/VNRC 5357 Zia Pueblo/VOH43ae/H99ae /// ss tester	11.9	**93.4**	19.3	3.5	50.0	55.8	88.4	5.1	1.3	52.8
03:407	Cuba164:S15-192-1-B-B/VNRC 5357 Zia Pueblo/VOH43ae/H99ae /// ss tester	9.1	**72.7**	17.8	4.5	50.6	57.1	90.5	8.0	1.2	45.5
03:408	Cuba164:S15-192-1-B-B/VCochiti Pueblo NRC 5298/VOH43ae/H99ae /// ss tester	9.5	**77.2**	16.9	5.2	49.3	58.1	91.9	8.9	1.8	60.8
03:410	Cuba164: S15-253-1-B-B/VNRC 5357 Zia Pueblo/VOH43ae/H99ae /// ss tester	10.8	**85.0**	20.3	4.6		50	78.1	2.0	24.0	50.3
03:411	Cuba164:S15-253-1-B-B/VNRC 5357 Zia Pueblo/VOH43ae/H99ae /// ss tester	11.8	**93.8**	19.1	5.6	52.0	57.3	90.5	3.0	4.1	55.5
03:412	Cuba164:S15-253-1-B-B/VNRC 5357 Zia Pueblo/VOH43ae/H99ae /// ss tester	11.6	**90.5**	19.7	3.1	50.7	53.8	85.3	3.2	1.8	
03:413	Cuba164:S2008a-469-1-B-B/VNRC 5357 Zia Pueblo/VOH43ae/H99ae /// ss tester	11.6	**92.4**	17.7	3.8	50.3	54.7	86.7	11.2	3.7	
03:421	AR01150:N04-213-1-B-B/VNRC 5357 Zia Pueblo/VOH43ae/H99ae /// ss tester	11.1	**87.9**	18.8	5.1	52.3	54.4	86.1	3.1	0.5	
	Ave. of OH43-type tester	**12.5**	**97.7**	**19.8**	**5.4**	**52.2**	**54.9**	**86.8**	**7.0**	**1.6**	
Check a	Proprietary amylomaize VII hybrid	8.8	**68.0**	21.2	3.4	51.9	51.5	81.7	5.3	3.8	
Check d	normal hybrid check	17.2	**125.6**	17.0	9.0		52.6	82.3	0.3	0.8	
	CV%		**19.5%**								
	LSD(0.05)		**30.6**								

Objective 3: Amylomaize VII single-cross hybrid evaluation using GEMS-0067 derived lines crossed among themselves:

In order to eliminate the need for parent lines owned by private seed companies, we are currently conducting a yield trial this summer (2006) to identify hybrids using publicly available GEM germplasm. The general principle, displayed in Figure 2, explains how the original GEMS-0067 line will be backcrossed to genetically distinct backgrounds that may combine well in single cross hybrids. Currently, 45 test hybrids are being evaluated in Novelty, MO and in Ames, IA.

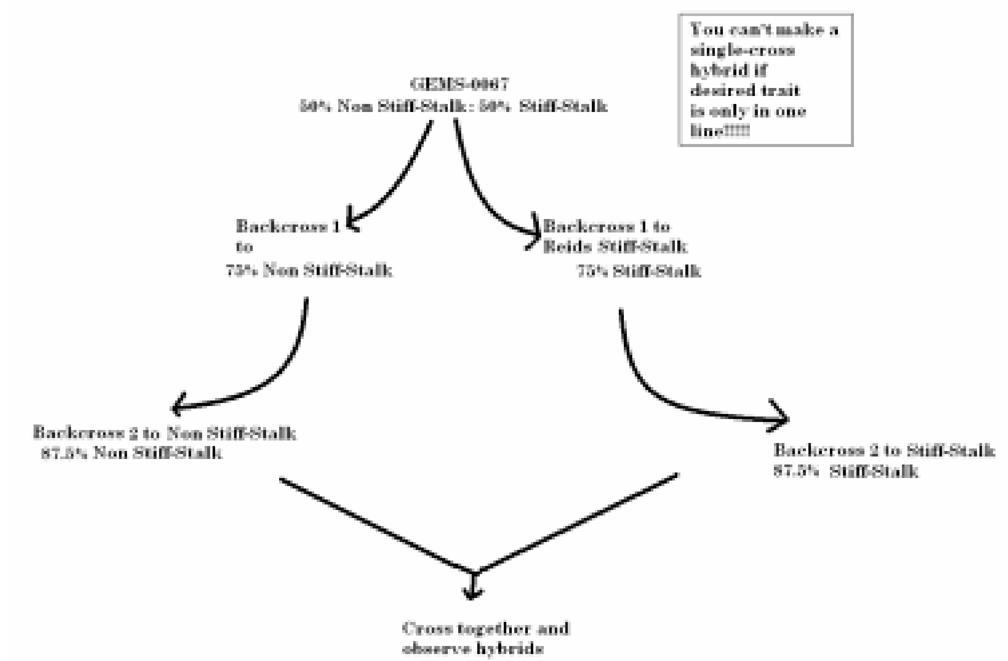


Figure 2. Development of experimental amylomaize VII hybrids using only publicly available germplasm currently (Summer 2006) growing in Novelty, MO and Ames, IA.

Conclusion:

This study is funded by the GEM (Germplasm Enhancement of Maize) project which is a cooperative effort of the United States Department of Agriculture (USDA) Agricultural Research Service (ARS), public universities, and industry. Currently, U.S. corn hybrids utilize less than five percent of world corn germplasm - this increases potential vulnerability to unforeseen pest problems, and may lead to an eventual yield cap. Exotic germplasm can provide genes for resistance to pests and increased yields, and may contain quality traits to meet new market demands. The Latin American Maize Project identified the top 268 corn accessions from among 12,000 gathered from the Western Hemisphere - GEM will enhance the best of these and other exotics for use by commercial corn-breeding programs in the U.S. GEM is supported by both the United States Congress and over 50 organizations and individuals from private industry and public institutions. Congress appropriates $500,000 per year to GEM. Cooperators do not contribute funds; rather, they donate their time and resources to the effort. This 'in-kind' support contributes an additional $450,000. As a public GEM cooperator, Truman State has focused on utilizing GEM germplasm for the development of high-amylose corn as previously discussed. It is hoped that local producers will recognize the importance of federally funded projects designed to provide economic opportunities for rural communities in Northeast Missouri.

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