Heritability and QTL for Popping Characteristics in Sorghum Grain

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Pop sorghum?

- Several companies already sell popped sorghum
- Increasingly popular with niche consumers





- Not the subject of the amount of selective breeding that popcorn has received
- Few releases of improved lines for popping (ex. SA389)

Objectives for this study....

- Determine the heritability of popping quality traits in *Sorghum*
- Determine the relative effects of genotype, environment, and genotype x environment for popping quality
- Identify QTL markers for popping quality

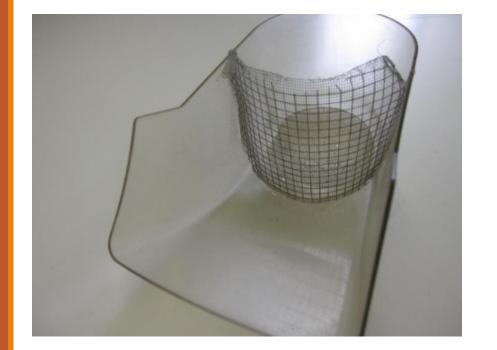
Population and Experimental Design

- RIL Population
 - Sureño x Tx430
 - Sureño: white endosperm, small kernel size, good popping quality
 - Tx430: yellow endosperm, large kernel size, poor popping quality
 - 130 RILs, F₁₂
- RCBD in the field with two repetitions
 - Weslaco 2012
 - Corpus Christi 2012
 - Halfway 2012

Phenotyping of Popping Traits

First...

- Heated airpoppers forpopcorn weremodified
- 500 seedwere countedfor each RIL
- Pre-popping volume was also taken (mL)





Next...

- Samples were heated for 2' 15"
- Two hot air poppers were blocked with each field rep
- Popped samples were poured on a 4.7mm sieve
- Kernels that fell through were considered unpopped





Finally...

- Volume (mL) of the popped material was taken
- Un-popped material was recounted
- Measurements a were used to calculate
 - Popping Efficiency (PE)
 - Expansion Ratio (ER)



Popping Efficiency

Expansion Ratio

$$\frac{(500-UPK)}{500} = PE$$

$$\frac{(PV)}{PE * UPV} = ER$$

UPK = Un-popped Kernels

PE = Popping Efficiency

PV = Popped Volume

UPV = Un-popped Volume

ER = Expansion Ratio

Heritability of Popping Characteristics

Statistical analysis

• Combined analysis (Random) across locations using SAS software, broad sense heritability was calculated on an entry mean basis:

$$H^{2} = \frac{\sigma_{g}^{2}}{\sigma_{g}^{2} + \frac{\sigma_{g}^{2}xe}{e} + \frac{\sigma_{e}^{2}}{re}}$$

Statistical model for combined analysis:

Y = Gen + Gen*Env + Rep(Env) + Environment + Popper(Rep*Env) + Error All effects considered random

Combined Analysis of Popping Traits Halfway, Corpus Christi, and Weslaco TX

Source	D.F.	M.S. (ER)	M.S. (PE)
Popper(Rep*Env)	3	7.7	0.08
Rep(Env)	6	12.0	0.47
Environment	2	110.2	3.27
Genotype	124	20.7	0.15
Geno*Env	246	6.6	0.05
Error	1003	2.3	0.01

PE: C.V. = 21.951 R-Square = .755

ER: C.V. = 22.068 R-Square = .602

p < .001 p < .01 p < .05

PE = Popping Efficiency

ER = **Expansion Ratio**

Entry Mean Heritability Estimates

	Heritability (H ²)		
Popping Efficiency	0.69(0.59-0.76)		
Expansion Ratio	0.70(0.62-0.77)		
Test Weight	0.77(0.69-0.82)		
Height	0.91 (0.88 - 0.93)		

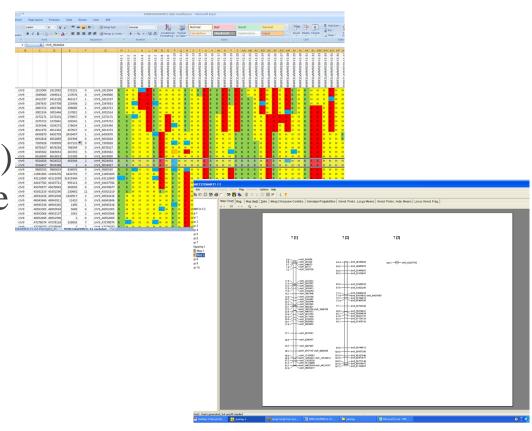
Conclusions...

- Popping quality traits are heritable within this population
- Improvement of popping traits in sorghum via selective breeding is likely possible

Quantitative Trait Loci

Genotyping and Linkage Map

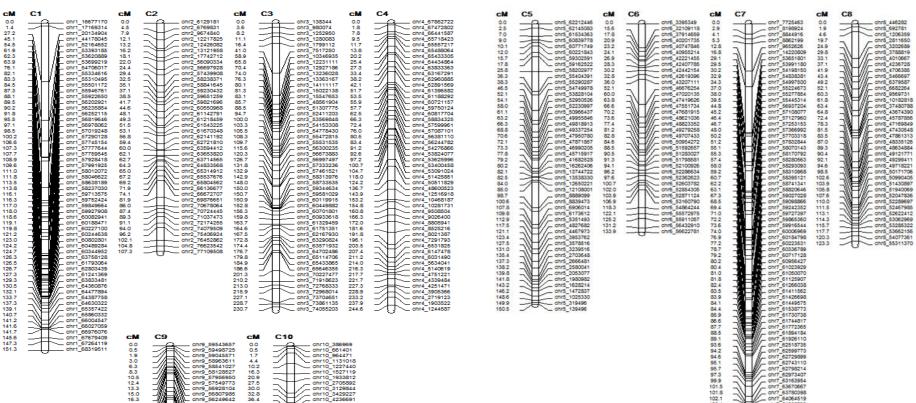
- Genotyping was conducted by the Klein laboratory using the Digital Genotyping (DG) methodology (Morishige et al. 2013)
- Genetic linkage map was constructed using JoinMap 4.0 software



Representative Pictures from Dr. Patricia Klein's laboratory: "Using Joinmap"

Morishige, D. T., Klein, P. E., Hilley, J. L., Sahraeian, S. M. E., Sharma, A., & Mullet, J. E. (2013). Digital genotyping of sorghum–a diverse plant species with a large repeat-rich genome. BMC genomics, 14(1), 448.

Linkage Map



CM	C9	CM	C10
0.0	chr9 59543657	0.0	chr10 386969
0.5	chr9_59498725	0.5	chr10_661401
1.9	_/ chr9_59045571	1.7	chr10_964471
3.0	_//	4.4	_/ chr10_1131018
6.3	_/// chr9_58541027	10.2	chr10_1227440
8.3	_// S	16.3	chr10_1527119
10.5	_///\	20.9	chr10_1933812
12.4	_/() chr9_57549773	27.5	chr10_2705892
13.3	_(M)	30.0	chr10_3129844
15.0	_////chr9_56807985	32.8	chr10_3429227
16.3	_///_ chr9_56249642	36.4	chr10_4236691
17.3	chr9_55703945	41.6	chr10_5123393
21.3	chr9_54719692	46.6	chr10_6259095
25.4	chr9_53507652	49.2	chr10_6840704
27.7	chr9_53326689	49.9	chr10_7422327
30.3	_M chr9_53013371	52.7	chr10_7766709
31.6	_W/\0\/\chr9_52906082	58.6	chr10_9359175
32.5 36.6		60.3 66.8	chr10_10372018 chr10_11278263
39.1		70.3	chr10 20158814
40.7	_XWXWA_ chr9 50963805	72.9	Chr10 44782293
47.3	AWIL WW chr9 50215869	74.7	Chr10 47851365
50.5	AWA NWA_ chr9 50064228	78.4	Chr10 48643124
54.9	WW chr9 49647362	80.1	Chr10 49561463
58.4	_AU	81.9	Chr10 49672819
60.9	_W chr9_48393924	84.1	_ chr10 50306985
65.6	_W chr9 47301112	87.2	_ chr10 51474072
70.1	_AVAI N.W chr9 40552899	91.0	_ chr10 52222389
73.9	_## 11693305	93.4	_ chr10 53231574
76.0	_////	95.4	_ chr10 53579630
84.0	_////\\\\\chr9_6835934	99.8	_ chr10 54095965
86.5	_//// 6280598	103.6	_ chr10_55184965
92.1	_////\\\\ chr9_5335038	106.3	chr10_55363444
96.0	_////	108.7	chr10_55552448
99.9	_////[_ chr9_4160516	112.0	chr10_56061985
102.2	_//// _ chr9_3558148	114.2	chr10_56486988
107.0	_J/// _ chr9_3127270	116.2	chr10_56748354
110.7	_//	119.1	chr10_56871290
113.6	J/	125.5	chr10_57617558
123.0	chr9_1990171	126.4	chr10_57690269
128.5	chr9_1557276	129.1	chr10_58025473
133.3	chr9_564242	131.2	chr10_58373800
		134.1	chr10_58987976
		135.2	chr10_59345589
		138.2	chr10_60223764
		140.0	chr10_60255444

	# of Markers	Length (cM)
Klein et al. (2001)	130	970
This Study	828	1527

QTL for Popping Efficiency

Environments	Chro	QTL 1 LOD Interval	QTL Peak Position (cM)	QTL Peak Position (Mbp)	LOD	R2	Add. Effect
CC	5	109.3 – 119.0	115.9	5.1	2.66	0.07	-0.05
HW	1	128.7 – 130.5	129.3	63.8	3.59	0.11	0.04
HW	9	0.0 - 1.9	0.0	59.5	6.05	0.18	-0.06
WE	2	7.0 - 11.7	7.9	8.2	3.57	0.11	0.06
WE	3	70.3 – 75.3	73.0	13.4	4.28	0.13	0.09

Positive effects correspond to the Tx430 allele; Negative effects correspond to the Sureño allele.

QTL for Expansion Ratio

Environments	Chro	QTL 1 LOD Interval	QTL Peak Position (cM)	QTL Peak Position (Mbp)	LOD	R2	Add. Effect
CC	3	85.5 – 95.0	91.5	52.7	3.49	0.11	-0.55
HW	5	66.1 – 68.4	67.0	49.7	5.23	0.15	-0.64
HW	10	49.1 – 51.0	49.9	6.4	4.25	0.13	-0.56
WE	9	11.1 – 12.9	11.9	57.8	5.17	0.15	-0.75

Positive effects correspond to the Tx430 allele; Negative effects correspond to the Sureño allele.

QTL for Popping Characteristics

- QTL for PE and ER were not consistent across multiple environments
- As expected, QTL for expansion ratio were all associated with the Sureño allele
- The lack of consistency of these QTL indicates that the traits are complex just as in popcorn

Acknowledgments

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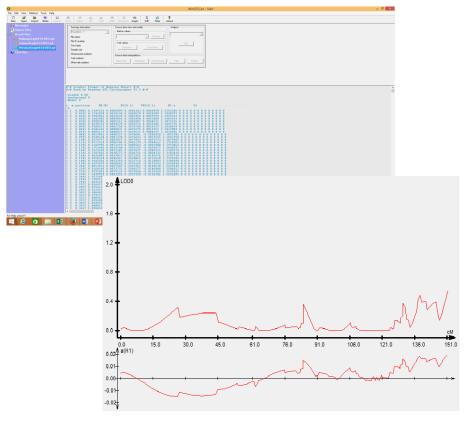
Questions?





QTL Mapping

- QTL were mapped using population means in WinQTL Cartographer 2.5 software
- Composite Interval Mapping with 1000 permutations at 0.05 significance level



Population Means

	Popping Efficiency (%)	Expansion Ratio (x : 1)	Kernel Diameter (mm)	Kernel Weight (mg)	Kernel Hardness (HI)
Sureño	39.4 a	9.8 a	2.5 a	25.1 a	96.4 a
Tx430	44.4 a	4.8 b	2.8 b	32.2 b	69.5 b
RILs	51.6 a	7.7 c	2.7 a	28.5 a	77.5 b
Range of RILs	6.1 – 87.9	2.9 – 13.2	2.2 - 3.2	18.8 – 40.4	20.3 – 101.0

	Test Weight (g/mL)	Plant Height (cm)	Flowering Date	Grain Color	Grain Mold
Sureño	0.79 a	161.9 a	84.5 a	1.7 a	2.3 a
Tx430	0.69 b	106.5 b	75.8 b	7.3 b	4.9 b
RILs	0.76 a	137.1 c	76.5 b	4.4 c	3.8 c
Range of RILs	0.57 - 0.84	73.7 – 234.5	56.5 – 91.5	1.0 – 9.0	2.0 - 6.5

Means followed by the same letter are not significantly different by Tukey's test (p < 0.05)

Pearson's Correlation Coefficients

	Popping Efficiency	Expansion Ratio
Popping Eff.	1.00	0.46***
Expansion Ratio	0.46***	1.00
Kernel Hardness	0.12**	0.18***
Kernel Diameter	0.42***	0.03
Kernel Weight	0.35***	-0.06
Endosperm Color	-0.24**	-0.22***

	Popping Efficiency	Expansion Ratio
Test Weight	0.13**	0.17***
Grain Mold	-0.16**	-0.15**
Plant Height	0.05	0.08*
Fat	-0.19***	-0.08*
Starch	0.15***	-0.01
Protein	-0.21***	0.05