HERITABILITY AND QUANTITATIVE TRAIT LOCI FOR POPPING CHARACTERISTICS IN SORGHUM GRAIN

A Thesis

by

NICHOLAS ACE PUGH

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Chair of Committee,	William L. Rooney
Committee Members,	Joseph M. Awika
	Patricia E. Klein
Head of Department,	David D. Baltensperger

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ABSTRACT

Popped sorghum (*Sorghum bicolor*, L. Moench) is becoming increasingly popular with niche consumers. However, sorghum has not undergone the years of intensive selective breeding that popcorn has. This study measured popping characteristics and grain traits to estimate heritability, the relative effect of environment and genotype x environment interactions on these traits and to identify quantitative trait loci (QTL) for popping quality. Using a heated-air popping methodology, a recombinant inbred line population was phenotyped for popping characteristics in grain from three environments in Texas. Entry-mean heritability of popping efficiency (PE) ranged from 0.595 – 0.755 and the heritability of expansion ratio (ER) ranged from 0.617 – 0.769 across environments. ANOVA indicate that both environment and genotype x environment interactions were significant sources of variation. Using genome sequence mapping technology, five QTL were identified for popping efficiency and four were identified for expansion ratio. Additionally QTL for endosperm color, kernel diameter, kernel weight, and kernel hardness were found, and several of those were consistent across multiple production environments. These results indicate that popping quality a complex quantitative trait in sorghum, but improvement of popping efficiency, expansion ratio, and other kernel characteristics via marker-assisted selection is possible.

DEDICATION

This scientific thesis is dedicated to the loving memory of my grandmother, Betty Pugh
It is my sincere hope that I can continue to make her proud.

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NOMENCLATURE

BLUP	Best Linear Unbiased Predictor
CC	Corpus Christi, TX
CIM	Composite Interval Mapping
DG	Digital Genotyping
ER	Expansion Ratio
HW	Halfway, TX
LOG	Log of Odds
MAS	Marker Assisted Selection
PCR	Polymerase Chain Reaction
PE	Popping Efficiency
QTL	Quantitative Trait Loci
RIL	Recombinant Inbred Line
SNP	Single Nucleotide Polymorphism
WE	Weslaco, TX

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1. INTRODUCTION

1.1 Sorghum Background

As one of the most drought tolerant of all the cereal grain crops, sorghum (*Sorghum bicolor* L Moench) is a staple food crop for millions of people living in Africa and Asia, but is comparatively underutilized as a human food source in other regions (Taylor et al. 2006). Traditionally used as animal feed in the United States, sorghum has become more popular as a food grain for several reasons. First, the identification of gluten intolerance has spurred an interest in gluten free grains. Since sorghum is one such grain, it has gained market share where gluten-free foods are attractive to consumers (Taylor et al. 2006). Second, consumer demand for GMO food products has enhanced food sorghum because there are no GMO sorghums in production. Finally, sorghum is an excellent source of many phenolic compounds, prized for its antioxidant content and usage in nutraceuticals and in functional foods (Dykes and Rooney 2006). Another potential niche market for the crop is popped sorghum, which is used primarily for the production of various confectionary treats.

1.2 Popped Grains

For as long as grain has been cultivated, a common method of processing has been popping. The popcorn (*Zea mays)* we recognize today is the result of systematic selection and breeding which have been remarkably successful in improving popping quality traits in corn. There are several of these quality traits that are critical to popcorn producers, including expansion ratio and popping efficiency (Song et al. 1991).

Commercial popcorn has a 95% or better popping efficiency as well as an excellent expansion ratio of 35:1 or more (Lyerly 1942; Pordesimo et al. 1991).

Like corn, grain sorghum is known to pop as well. While several studies have been conducted on the popping quality of sorghum, these studies have mostly evaluated varieties adapted and produced in Asia (Murty et al. 1982; Murty et al. 1988). Reports of the popping quality of sorghums grown in the United States are very limited (Gaul and Rayas-Duarte 2008; Rooney and Rooney, 2013). The production and distribution of popped grain other than *Zea mays* is gaining increased interest with companies such as "Just Poppin®" and "Mini Pops®", particularly those interested in appealing to niche consumers looking for something new to eat. The primary focus for popped sorghum is now the production of treats in the form of cookies or bars; in fact, popped sorghum may even be superior for this purpose to popcorn due to its smaller size (Gaul and Rayas-Duarte 2008).

Reports on the popping ability of sorghum grain typically evaluate sorghum varieties and hybrids developed for other uses because there has not been any systematic breeding to improve the popping qualities of grain sorghum. Popcorn has benefitted from years of careful selection for traits that improve popping quality, while studies focused on popped sorghum remain relatively scarce. Rooney and Rooney (2013) reported that sorghum can have similar popping efficiency but a lower expansion ratio than popcorn and they also identified genotypes that have desirable popping characteristics (Rooney and Rooney 2013). The ability to identify genotypes that are superior for the popping traits ensures that selection for those quality traits is possible;

however, it is also necessary that those traits be heritable. Popping quality traits, such as expansion ratio, are strongly heritable in corn which serves as an excellent impetus to explore the same possibility in sorghum grain (Crumbaker et al. 1949). Crumbaker et al. (1949) demonstrated that low popping volume was partially dominant over high popping volume and that there was a relationship between kernel starchiness and popping volume.

1.3 Popping Mechanism

There are numerous factors that may influence popping quality in grain and to understand these factors, it is important to understand the process itself. Popping occurs when moisture in the center of the endosperm vaporizes and increases the pressure in the endosperm enough to rupture and burst the outer endosperm (Hoseney et al. 1983). The composition of the endosperm is critical to the quality of the resulting popped grain (Pordesimo et al. 1991). In corn, endosperm starch composition as well as pericarp thickness are both important factors; a thicker pericarp and thus greater kernel hardness is desirable (da Silva 1993). Endosperm composed mostly of hard starch has superior popping efficiency to endosperm composed of softer starch in corn (Wilier 1927) and thicker pericarp was a strong predictor of expansion ratio in microwave popcorn (Mohamed et al. 1993). Other physical characteristics that may affect popping quality include the type of endosperm and its structure and grain density. Damage to individual kernels prior to popping can hinder the buildup of interior vapor pressure necessary to produce the rupture of the pericarp that would result in a desirable, fully popped kernel

(Singh et al. 1997). As such, factors originating in the field such as grain weathering can also play a large role in the quality of the resulting popped grain.

1.4 Heritability of Popping Quality

Popcorn breeders have systematically improved popping quality over many years. Improvement was possible because these traits were heritable to some extent (Crumbaker et al. 1949). To improve sorghum for popping ability, it is important to assess the variation in popping efficiency and expansion ratio and determine if these traits are selectable.

1.5 Quantitative Trait Loci

If popping efficiency (PE) and expansion ratio (ER) are heritable in sorghum as they are in corn, a selective breeding approach for pop sorghum is needed that is similar to that used in popcorn. In addition, breeding efficiency is increased when molecular tools are available; to that end, identifying QTL markers for PE and ER should improve the efficiency of a pop sorghum breeding program just as in maize (Collard et al. 2005; Lu 2003). Previously, QTL have been identified for popping quality traits and related traits in maize (Lu 2003; Li 2007); and thus it is conceivable that a similar approach would be successful in sorghum. Sorghum has a fully sequenced genome (Paterson et al. 2009), and sorghum improvement programs have benefitted greatly from advances in genotyping technology. One of the newest and most effective techniques is that of digital genotyping (DG). This technique can be used to quickly generate very accurate genotyping data which can then be used for a wide variety of analyses, including QTL mapping (Morishige et al. 2013). This DG technology was used in conjunction with a

standard QTL mapping methodology in order to identify regions of the genome that affect PE and ER in sorghum.

Rooney and Rooney (2013) identified two lines that differed for popping ability. These lines were similar in lineage to RTx430 and Sureño (Rooney, personal communication) for which an RIL population has been developed and used to map grain mold resistance in sorghum (Klein et al. 2001). Due to the large differences between the two parents of this RIL population, which were Sureño (Meckenstock et al. 1993) and RTx430 (Miller 1984), progeny were expected to segregate for many traits of interest including PE and ER (Rodriguez-Herrera 2001).

Within this context, the goal of this study was to investigate the genetic and environmental factors that affect and/or are associated with popping of grain sorghum. Specifically, the objectives of this study were: (1) to determine the relative genetic and environmental factors affecting the heritability of popping efficiency and expansion ratio and related traits in sorghum; and (2) to identify QTL associated with popping efficiency, expansion ratio and related traits in grain sorghum. Information on the relative heritability of these traits and available QTL markers can be used to bolster the effectiveness of a popping quality improvement program (Collard et al. 2005).

2. HERITABILITY OF POPPING CHARACTERISTICS IN SORGHUM GRAIN

2.1 Introduction

As one of the most drought-tolerant of all the cereal grain crops, sorghum (*Sorghum bicolor* L Moench) is a staple food crop for millions of people living in Africa and Asia, but is comparatively underutilized as a human food source in other production regions (Taylor 2006). Traditionally used as animal feed grain in the United States, food grade sorghum is becoming more popular for several reasons. Sorghum is gluten free, making it desirable in the gluten-free food market (Taylor 2006). Second, consumer demand for GMO food products has enhanced food sorghum because there are no GMO sorghums in production. Finally, sorghum is an excellent source of many phenolic compounds, prized for its antioxidant content and usage in nutraceuticals and in functional foods (Dykes and Rooney 2006).

Another potential niche market for the crop is popped sorghum, which given its size has application in various confectionary treats. The production and distribution of popped grain other than *Zea mays* is gaining increased interest with companies such as "Just Poppin®" and "Mini Pops®", particularly those interested in appealing to niche consumers looking for something new to eat. The primary focus for popped sorghum is now the production of treats in the form of cookies or bars; in fact, popped sorghum may even be superior for this purpose to popcorn due to its smaller size (Gaul and Rayas-Duarte 2008).

For as long as grain has been cultivated, a common method of processing has been popping. The popcorn (*Zea mays)* we recognize today is the resulted for systematic

selection for popping efficiency and eventually expansion ratio. Once breeding techniques were developed, these programs have been remarkably successful in improving popping quality traits in corn (Song et al., 1991). Popcorn produced and processed today has at least a 95% or better popping efficiency (PE) as well as an excellent expansion ratio (ER) of at least 35:1 or greater (Lyerly 1942; Pordesimo et al. 1991).

Several studies have been conducted on the popping quality of sorghum, but these studies have mostly evaluated Asian varieties (Murty et al. 1982; Murty et al. 1988). In Murty et al. (1988), it was determined that ER was governed by both dominance and additive gene effects and that there were also significant dominance x dominance interaction effects involved. Any reports of the popping quality of sorghums grown in the United States are very limited (Gaul and Rayas-Duarte 2008; Rooney and Rooney, 2013). Gaul and Rayas-Duarte (2008) demonstrated that sorghum with a thicker pericarp had a superior PE and ER. Rooney and Rooney (2013) reported that sorghum can have similar PE but a lower ER than popcorn. It was also possible to identify genotypes that have desirable popping characteristics (Rooney and Rooney 2013).

There are numerous factors that may influence PE and ER in grain sorghum based on knowledge from popcorn studies (Karababa 2006). To understand these factors, it is important to understand the process itself. In any cereal grain, popping occurs when moisture in the center of the endosperm vaporizes and increases the pressure in the endosperm enough to rupture and burst the outer endosperm (Hoseney et al. 1983). The composition of the endosperm is critical to the quality of the resulting

popped grain (Pordesimo et al. 1991). Endosperm starch composition as well as pericarp thickness are both important factors; a thicker pericarp and thus greater kernel hardness is desirable (da Silva 1993). Endosperm composed mostly of hard starch has superior popping capacity compared to endosperm composed of softer starch in corn (Wilier 1927). In addition, thicker pericarp has been a strong predictor of expansion ratio in microwave popcorn (Mohamed et al. 1993). Other physical characteristics that may affect popping quality include the type of endosperm and its structure and grain density. Damage to individual kernels prior to popping can hinder the buildup of interior vapor pressure necessary to produce the rupture of the pericarp that would result in a desirable, fully popped kernel (Singh et al. 1997). As such, factors originating in the field such as grain weathering can also play a large role in the quality of the resulting pop sorghum.

Given the lack of market, breeding for popping sorghum has been limited. Most research has evaluated sorghum varieties and hybrids developed for other uses. Popcorn has benefitted from years of careful selection for traits that improve popping quality, and it is logical to assume that the same would happen with sorghum. The ability to identify genotypes that are superior for the popping traits ensures that selection for those quality traits is possible; however, it is also necessary that those traits be heritable. Popping quality traits, such as expansion ratio, are strongly heritable in corn which serves as an excellent impetus to explore the same possibility in sorghum grain (Crumbaker et al. 1949).

The objectives of this study are: (1) to assess the relative effects of genotype, environment and genotype x environment on the popping characteristics of sorghum and; (2) to determine the heritability of these popping characteristics.

2.2 Materials and Methods

2.2.1 Plant Germplasm

A recombinant inbred line (RIL) population of 130 entries was derived from the cross of Sureño x RTx430 (Rodriguez-Herrera et al., 2000). Sureño has a pedigree of [(SC423 x CS3541) x E35-1]-2 and was released for its superior grain quality and grain weathering characteristics (Meckenstock et al., 1993). RTx430 has a pedigree of (Tx2536 x SC170-6-5-1)-10-4-4-1-4 and was released based on its excellent general combining ability in hybrids and disease resistance (Miller, 1984). Previous work by Rooney and Rooney (2013) described differences in popping quality between derivatives of Sureño (Meckenstock et al. 1993) and Tx430 (Miller 1984); with Sureño and RTx430 derivatives having good and poor popping characteristics respectively.

The 130 RILs and the parents were planted in replicated trials in three locations (Weslaco, Corpus Christi, and Halfway, Texas) in 2012. The test was planted in a randomized complete block design (RCBD) layout. Plants grown in the Corpus Christi (CC) environment were grown in Orelia clay loam with the following rainfall: March = 3.43 cm, April = 6.55 cm, May = 7.49 cm, June = 3.91 cm, and July = 3.40 cm. Plants grown in the Halfway (HW) environment were grown in Pullman clay loam with the following rainfall: May = 2.69 cm, June = 8.53 cm, July = 1.22 cm, August = 2.18 cm). Plants grown in the Weslaco (WE) environment were grown in Hidalgo sandy clay with the following rainfall: February = 7.01 cm, March = 1.02 cm, April = 0.43 cm, May =

4.55 cm, June = 1.47 cm). Standard agricultural practices for sorghum grain were used in each location.

2.2.2 Phenotypic Trait Evaluation

Data was collected in the field for several agronomic traits that could influence grain quality. Days to flowering was recorded as the number of days from planting to when 50% of the plants reached mid-anthesis. Plant height (cm) was measured as the distance from the tip of the panicle to the base of the plant and it was recorded just prior to harvest. Grain weathering is a subjective visual rating of the damage caused to grain due to exposure to the environment (i.e., insects, pathogens, and climate) (Williams and Rao 1981; Rodriguez-Herrera et al. 2000; Klein et al. 2001). This measurement was before the grain was harvested, with a rating of 1 corresponding to clean grain with no weathering and a 9 corresponding to grain that was completely weathered and/or destroyed.

In each environment, grain was harvested soon after black layer (physiological maturity) to minimize the effect of post-maturity grain weathering. From each plot a minimum of five panicles were harvested from each experimental unit and if the panicles were small, additional panicles were harvested to ensure large enough grain quantity. These panicles were then threshed in bulk using a single head thresher (Alamaco) that used AC/DC power. To remove any remaining glumes or panicle residue, the grain was cleaned using a Wintersteiger LD180 (Wintersteiger Ag; Ried, Austria). The threshed and cleaned grain was packaged and stored at 10° C.

The grain was evaluated for several traits. To estimate starch, fat, ash, protein and fiber concentrations, unprocessed grain samples from each entry in each location were scanned using a FOSS XDS NIR system and the scans were converted to percent estimates of each compound using standards developed by the Texas A&M Agrilife Sorghum Improvement laboratory. Since RTx430 (yellow) and Sureño (white) differed for endosperm color, a subjective visual rating (1, white to 9, yellow) for endosperm color was taken to accompany the results of the colorimetry process. Kernel hardness was measured using a Single Kernel Hardness Tester, model 4100 (Perten Instruments) where 300 individual kernels were crushed separately, calculating a mean and standard deviation. The machine also took measurements of individual kernel weight and diameter which were also used for correlation analyses.

Popping quality for each entry was measured by estimating both PE and ER. A set of modified heated air poppers (Presto 04821) were outfitted with steel wire mesh to keep the sorghum kernels from being ejected during the popping process. Each experimental unit was popped twice using two different poppers and each replication was blocked according to their respective air poppers to partition the effect of the individual poppers into the replication effect. While optimum moisture content for popping in *Zea mays* is about 14-16% moisture (Gökmen 2004) and Rooney and Rooney (2013) adjusted moisture content of their samples to 15%, it was not feasible to adjust moisture content due to the sample numbers and size of the popped sample. Samples that were popped in this study averaged 11% moisture with a range from 10-12% moisture content.

For each test pop, 500 seed were counted using an OLD MILL electronic seed counter Model 850-3. Prior to popping sample seed volume (mL) was measured using a graduated cylinder and the weight of the grain was measured in grams. Each sample was placed in the popper which was run for 2 minutes and 15 seconds which was determined to be the optimum time for popping. Immediately after popping, the sample was poured onto a 4.7 mm sieve and shaken. Any kernels that fell through the sieve were considered un-popped kernels and any that remained on the sieve were considered popped kernels. The volume of the popped fraction was measured in a graduated cylinder. The un-popped kernels were collected and recounted using the OLD MILL electronic seed counter Model 850-3.

Using information collected, popping efficiency (PE) was calculated as the percentage of popped kernels divided by the total kernels popped (500). Popping efficiency was calculated using the formula: $\frac{(500-UPK)}{500} = PE$ where UPK is the number of unpopped kernels remaining after popping. Expansion Ratio is a ratio of the volume of the popped grain divided by the volume of the unpopped grain and was calculated using the original volume (mL) of 500 seed (UPV) and the popped volume (mL). The formula for Expansion Ratio is $\frac{(PV)}{PE*UPV} = ER$.

2.2.3 Statistical Analysis

Data analysis was conducted using SAS 9.2 software. All dependent variables were analyzed by environment using an all random model of $Y = \alpha_i + \beta(\alpha)_{ij} + y_k + \alpha y_{ik} + \delta(\beta \times \alpha)_{lij}, + \varepsilon$ where α= environments (i = 1, 2, 3), β = repetitions (j = 1, 2), y =

genotypes (k = 1… 130), δ = poppers (l = 1, 2), and ε = error. Variance components were estimated from this analysis to calculate Heritability (H^2) on an entry mean basis using the formula $h^2 = \frac{\sigma_G^2}{\sigma_p^2}$. A Bartlett's test of Homogeneity detected significant variation among error terms from the individual environments, but transformation failed to reveal a means of adjusting to the data to eliminate this problem and there was no phenotypic reason. Variance components were estimated from these analyses to calculate broad-sense Heritability (H^2) on an entry mean basis using the formulas $h^2 = \frac{\sigma_G^2}{\sigma_p^2}$ and $H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{gxe}^2}{e} + \frac{\sigma_\varepsilon^2}{re}}$. Additionally, confidence intervals were calculated for these heritability estimates using the procedure described by Knapp et al. (1985). As appropriate, PROC CORR was also used to determine the relationship between the traits and examine them for correlations to popping quality.

2.3 Results and Discussion

2.3.1 Population Means and Variation

The RIL population segregated for all traits evaluated as did RTx430 and Sureño (Table 1). Interestingly, the mean PE for Sureño was actually lower in two of the locations (Corpus Christi and Halfway) than the RTx430 parental line. This is contrary to prior reports of derivatives of Sureño that were superior to RTx430 derivatives for PE (Rooney and Rooney, 2013). While Sureño exhibited worse PE in two locations than RTx430, it performed much better in the other location (Weslaco). There are two possible explanations. First, Rooney and Rooney (2013) reported on derivatives of Tx430 and Sureño so there may be inherent differences from those lines to the lines

tested herein. Second, RTx430 has larger seed size and if the grain split during the popping process, the grain was large enough that these lightly popped kernels had expanded enough to stay above the sieve. If this were the case, the ER would be reduced in RTx430 and this was observed as Sureño had consistently higher ER than RTx430 across all environments (Table 1). For both traits, there was a high degree of transgressive segregation in the progeny (Table 1). For the agronomic, kernel and composition traits, differences as expected between the parents were detectable (ie, plant height, kernel diameter, and endosperm color) and the RIL population means were between the parents with transgressive segregation present for every measured trait with the exception of many of composition traits. For several of these, the RIL means were actually higher than either parent although individual RILS were lower than the lowest parent in all cases (Table 1). In many cases, there were no differences between the two parents indicating a minimal range which would allow the RIL progeny to differ from midparent values.

2.3.2 Analyses of Variance

Significant differences due to genotypes were detected for every measured trait in the combined analysis (Table 2). In addition, environment and genotype x environment interactions were significant and had larger relative effects than genotype for all traits except moisture (Table 2). Because grain samples were harvested and allowed to dry prior to analysis, the samples simply equilibrated to a standard moisture level for grain which is approximately 11%. Thus, no differences in moisture were expected even though genotypes may have differed at harvest. Overall,

Table 1: Summary of Population Means. Summary of population means obtained from a population of RILs derived from a cross between Sureño x RTx430. Three locations were recorded (CC, HW, and WE in Texas). The mean for the two parents and their progeny are shown, as well as the range for the progeny from lowest to highest value. Means followed by the same letter are not significantly different by Tukey's test ($p < 0.05$).

Trait	Line	CC	HW	WE
Popping Efficiency (%)	Tx430	45.6 A	57.4 A	30.2 A
	Sureño	39 A	29.9 B	49.4 B
	RIL	44.9 (12.6 - 83.7) A	61.3 (24.7 - 87.9) A	48.5 (6.1 - 80.3) B
Expansion Ratio (x : 1)	Tx430	4.8 A	5.7 A	3.9 A
	Sureño	9.3 B	10.6 B	9.5 B
	RIL	7.6 (4.8 - 13.2) B	8.3 (4.9 - 12.8) B	7.3 (2.9 - 12.2) C
Plant Height (cm)	Tx430	100.3 A	103.6A	115.6 A
	Sureño	151.1 B	139.7 B	195.1 B
	RIL	128.5 (78.7 - 188.0) AB	122.2 (73.7 - 174.0) AB	160.5 (85.1 - 234.5) B
Test Weight (g / mL)	Tx430	0.69 A	0.69 A	0.69 A
	Sureño	0.78 B	0.77 B	0.82 B
	RIL	0.76 (0.57 - 0.79) B	0.74 (0.67 - 0.79) B	0.78 (0.60 - 0.84) B
Flowering Date	Tx430	67.3 AB	72.8 A	87.3 A
	Sureño	76.5 A	90.5 B	86.5 A
	RIL	68.3 (56.5 - 81.0) B	77.7 (70.5 - 90.5) C	83.5 (71.0 - 91.5) A
Grain Mold	Tx430	4.0 AB	Not Scored	5.8 AB
	Sureño	2.5 A	Not Scored	2.0 A
	RIL	3.9 (2.5 - 6.0) B	Not Scored	3.7 (2.0 - 6.5) B
Endosperm Color	Tx430	7.5 A	8.5 A	6.0 A
	Sureño	1.3 B	2 B	1.8 B
	RIL	4.7 (1.0 - 9.0) C	4.0 (1.0 - 9.0) B	4.5 (1.0 - 8.0) C
Kernel Weight (mg)	Tx430	34.1 A	35.3 A	27.06 A
	Sureño	22.6 B	24.8 B	27.8 A
	RIL	26.8 (20.6 - 35.2) B	29.9 (21.5 - 39.2) B	28.9 (18.8 - 40.4) A
Kernel Diameter (mm)	Tx430	3.0 A	2.9 A	2.6 A
	Sureño	2.4 B	2.4 B	2.6 A
	RIL	2.6 (2.2 - 3.0) B	2.7 (2.4 -3.1) B	2.7 (2.3 - 3.2) A

Table 1 continued.

Trait	Line	CC	HW	WE
Kernel Hardness	Tx430	69.6 A	73.9 A	64.9 A
	Sureño	97.2 A	96.8 B	95.3 B
	RIL	78.1 (44.5 - 101.0) A	75.9 (27.3 - 98.7) A	78.5 (20.3 - 100.6) AB
Fat	Tx430	2.19 A	2.34 AB	2.75 AB
	Sureño	2.29 AB	1.89 A	2.56 B
	RIL	2.37 (1.71 -2.84) B	2.28 (1.64 - 2.84) B	2.93 (1.85 - 3.69) A
Fiber	Tx430	1.83 A	1.59 A	1.83 AB
	Sureño	1.94 A	1.52 A	1.97 A
	RIL	1.84 (1.54 -2.08) A	1.79 (1.65 - 2.00) A	1.85 (1.58 - 2.05) B
Ash	Tx430	1.33 A	1.39 A	1.24 A
	Sureño	1.40 A	1.69 A	1.32 A
	RIL	1.36 (1.28 - 1.44) A	1.38 (1.29 - 1.45) A	1.29 (1.15 - 1.42) A
Starch	Tx430	64.87 A	66.18 A	65.57 A
	Sureño	64.59 A	66.31 A	65.21 A
	RIL	65.08 (63.40 - 66.31) A	66.01 (64.42 - 67.64) A	66.03 (63.7 - 68.5) A
Protein	Tx430	12.49 A	10.93 A	11.36 A
	Sureño	13.66 A	11.71 A	12.52 A
	RIL	12.87 (10.02 - 14.91) A	11.18 (9.85 - 12.42) A	11.19 (7.8 - 14.1) A
Moisture	Tx430	12.11 A	11.87 A	10.89 A
	Sureño	11.23 B	11.71 A	10.43 A
	RIL	11.42 (9.55 - 12.41) B	11.85 (10.78 - 12.87) A	10.99 (8.7 - 12.2) A

moisture equilibration was probably beneficial to the study as moisture is important in popping quality and was effectively removed as a confounding variable.

In popcorn, PE and ER are influenced by numerous factors such as kernel size, kernel weight, and other similar traits (Karababa 2006). Each of these traits are influenced by environmental factors like those seen in this study. Specifically, the HW environment was superior to the other two environments for both PE and ER in sorghum. Thus, the production environment remains the largest single effect on grain sorghum popping traits. In comparison of the relative magnitude of the genotype and genotype x environment effect, the genotype effect was roughly twice that of the GxE effect. This is comparable to reports from other studies and indicates a trait that will likely be responsive to selection but with care to identify optimum environments for specific genotypes.

Table 2: Analysis of Variance. Analysis of Variance (ANOVA) for various grain quality traits taken from a population of RILs derived from a cross between Sureño x RTx430. These Mean Square (M.S.) values were calculated after combining the phenotypic data across three environments (CC, HW, and WE in Texas).

Source	D.F.	Test Weight	Seed Diameter	Kernel Hardness	Plant Height	Flowering Date	Endosperm Color
Rep (Environment)	3	17.705**	0.0409**	81.413	120.861***	63.611***	53.496***
Environment	2	592.347***	0.8519***	347.537***	16424.422***	14607.206***	2.0366
Genotype	124	18.668***	0.0818***	361.440***	593.228***	75.693***	8.9497***
Genotype*Environm	247	4.810*	0.0132**	84.871***	54.448***	18.983***	1.9739***
Error	353	3.935	0.0096	37.008	15.992	10.698	1.2913
R^2		0.775	0.854	0.872	0.953	0.917	0.792
C.V. (%)		3.355	3.667	7.859	7.401	4.274	25.314

Source	D.F.	Fat	Fiber	Ash	Starch	Protein	Grain Mold
Rep (Environment)	3	0.0291	0.0155*	0.0155***	0.8576	1.1476	0.3527
Environment	2	31.268***	0.2128***	0.4943***	68.756***	222.497***	53.8245***
Genotype	124	0.2135***	0.0216***	0.0053***	1.747***	2.243***	1.7789***
Genotype*Environm	247	0.1019***	0.0110***	0.0029**	1.0192***	1.297***	1.1046*
Error	353	0.0508	0.0055	0.0021	0.5023	0.614	0.809
R^2		0.864	0.754	0.764	0.773	0.828	0.677
C.V. (%)		8.356	4.05	3.398	1.079	6.659	24.867

* significant at $p < 0.05$
** significant at $p < 0.01$
*** significant at $p < 0.00$

Table 3: Analysis of Variance (Popping Traits). Analysis of variance table containing mean squares (M.S.) for both popping quality traits. These values were obtained via a combined analysis across all three locations used in the study (CC, HW, and WE in Texas).

Source	D.F.	Popping Efficiency	Expansion Ratio
Rep (Environment)	3	0.4746***	12.0105**
Popper(Rep*Environment)	6	0.0767***	7.6766*
Environment	2	3.2656***	110.1905***
Genotype	124	0.1549***	20.6405***
Genotype*Environment	246	0.0472***	6.5908***
Error	1003	0.01282	2.9701
R^2		0.755	0.602
C.V. (%)		21.951	22.068

* significant at $p < 0.05$
** significant at $p < 0.01$
*** significant at $p < 0.001$

2.3.3 Heritability Estimates

Heritability across all traits ranged from a low of 0.687 to a high of 0.908 (Table 4). As expected, heritability of plant height was very high. For flowering date, H^2 estimates were moderately high but lower than plant height (Table 4). The genetic basis for both traits are well established and these estimates are consistent with expectations. Broad-sense heritability estimates were moderate to high for both PE and ER (Table 4). Previous studies in maize have concluded that popping quality is highly heritable within popcorn (Crumbaker et al. 1949; Robbins and Ashman 198). The actual popping quality of any given line can easily increase or decrease depending on the location in which it is

grown, and improvement can easily be more or less difficult for varying populations depending on the environment in which the improvement is attempted. Despite these potential caveats, it is clear that the popping traits were heritable within this population. Heritabilities for kernel traits were relatively high, indicating selection for these traits should be effective. Heritability estimates have been moderate to high for kernel characteristics such as hardness, seed size, and seed weight in other studies in sorghum grain (Voigt et al. 1966; Ibrahim et al. 1985).

Table 4: Entry Mean Heritability Estimates. Entry-mean heritability (H^2) estimates for various popping and popping-related traits. Confidence intervals are provided in parenthesis (0.05 – 0.95). These values were calculated using phenotypic traits taken from a population of RILs derived from a cross between Sureño x RTx430. Heritability estimates were calculated across three environments (CC, HW, and WE in Texas).

	Heritability (H^2)
Expansion Ratio	0.704 (0.617 - 0.769)
Popping Efficiency	0.687 (0.595 - 0.755)
Test Weight	0.765 (0.696 - 0.816)
Endosperm Color	0.807 (0.751 - 0.849)
Plant Height	0.908 (0.880 - 0.928)
Flowering Date	0.739 (0.662 - 0.796)
Kernel Hardness	0.766 (0.696 - 0.817)
Kernel Diameter	0.838 (0.789 - 0.874)
Kernel Weight	0.868 (0.829 - 0.897)

2.3.4 Correlation Analysis

Significant correlations were detected between various grain quality traits and popping traits in this population, but no single correlation was completely predictive of popping quality (Table 5). The correlation between ER and PE (r=.0.46) was significant, but not as strong as that reported by Rooney and Rooney (2013). In comparison, this study evaluated a much larger set of germplasm. Other traits involved in significant correlations included test weight, endosperm color, fat content, ash, starch, protein, kernel hardness, kernel diameter, and kernel weight. Endosperm color was negatively correlated with both PE and ER meaning that genotypes with a more yellow endosperm tended to have lower PE and ER. This is because higher color scores corresponded to yellower endosperm. While this fits with the parental observations, it does not necessarily indicate that all yellow endosperm genotypes have low potential popping.

A strong positive correlation between popping characteristics and both kernel weight and diameter, indicates that larger kernels have better popping characteristics. This is despite the fact that the parent with larger kernel diameter was Tx430 which is the poorer popping line. In maize, the relationship between kernel diameter or kernel size and popping quality has been inconsistent with independent studies reporting positive and negative correlations as well as a lack of any correlations at all between the traits (Lin and Anantheswaran 1988; Pordesimo et al. 1991; Allred-Coyle et al. 2000; Tian et al. 2001; Ceylan and Karababa 2002; Karababa 2006). Karababa (2006) reconcile this inconsistency in corn with the conclusion that there is middle range for kernel size that is most suitable for popping in corn. It remains to be seen if the same is

observed in sorghum. Kernel hardness had a positive correlation (Table 5) with both popping traits, but its correlation was not as strong as that reported in popcorn (da Silva 1993). This indicates that a line with both large seed size and high seed weight would be optimal, assuming that the phenotyping method effectively adjusts for seed size if screens are used to quantify PE. Given the absence of a single correlation that is predictive, a series of correlative traits might aid in selection.

Table 5: Pearson's Correlation Coefficients. Pearson's correlation coefficients for various popping and popping related traits. These values were calculated using measurements obtained from a RIL population derived from a cross between Sureño x RTx430. Data was combined across three locations (CC, HW, and WE in Texas)

	Popping Efficiency	Expansion Ratio
Popping Efficiency	1.0000	0.4584***
Expansion Ratio	0.4584***	1.0000
Test Weight	0.1331**	0.1686***
Plant Height	0.0503	0.0779*
Grain Mold	-0.1592**	-0.1543**
Endosperm Color	-0.2407**	-0.2218***
Fat	-0.1924***	-0.0753*
Fiber	0.0229	0.0324
Ash	0.1635***	0.0098
Starch	0.1535***	-0.0119
Protein	-0.2138***	0.0461
Kernel Hardness	0.1152**	0.1759***
Kernel Diameter	0.4237***	0.0328
Kernel Weight	0.3466***	-0.0587

* significant at $p < 0.05$
** significant at $p < 0.01$
*** significant at $p < 0.001$

2.3.5 Top Performing RILS

Several of the RILs used for this heritability study demonstrated improved PE, ER and agronomic combinations and would be candidates for selection for popping (Table 1, Table 6). For PE, RIL 065 was a consistently good performance in in every environment, and was the top performing line in HW (Table 6). Other excellent RILs were RIL 107, RIL 139, RIL 121 and RIL 103 (Table 6). For ER, RIL 065 was in the top 10% across environments, but was not the best in any environment (Table 6). RIL 017 also stands out as an excellent line that performed well in every environment, even performing the best in WE (Table 6). Other notable lines include RIL 081, RIL 094, RIL 070, RIL 129, and RIL 075 (Table 6). In the WE environment the Sureño parent performed better than many of the RILs, in contrast to the population's otherwise large amount of transgressive segregation (Table 1, Table 6).

Table 6: Top Ten Performing RILs. Top ten performing RILs from a population derived from a cross between Sureño x RTx430. Three environments are represented, CC = Corpus Christi, HW = Halfway, and WE = Weslaco in Texas.

	CC	HW	WE
Popping Efficiency	RIL 103 (0.84)	RIL 065 (0.88)	RIL 129 (0.80)
	RIL 139 (0.78)	RIL 121 (0.87)	RIL 065 (0.79)
	RIL 107 (0.77)	RIL 107 (0.86)	RIL 019 (0.77)
	RIL 027 (0.75)	RIL 035 (0.84)	RIL 139 (0.76)
	RIL 065 (0.75)	RIL 023 (0.81)	RIL 103 (0.75)
	RIL 069 (0.73)	RIL 137 (0.81)	RIL 031 (0.74)
	RIL 035 (0.69)	RIL 098 (0.81)	RIL 117 (0.73)
	RIL 074 (0.68)	RIL 019 (0.80)	RIL 124 (0.72)
	RIL 091 (0.67)	RIL 092 (0.79)	RIL 028 (0.71)
	RIL 050 (0.66)	RIL 135 (0.79)	RIL 121 (0.71)
Expansion Ratio	RIL 081 (13.18)	RIL 070 (12.77)	RIL 017 (12.22)
	RIL 094 (12.58)	RIL 129 (12.51)	RIL 075 (11.67)
	RIL 065 (12.02)	RIL 017 (11.92)	RIL 010 (11.64)
	RIL 042 (10.88)	RIL 065 (11.73)	RIL 119 (11.64)
	RIL 103 (10.86)	RIL 023 (11.06)	RIL 086 (11.60)
	RIL 129 (10.72)	RIL 019 (11.01)	RIL 093 (11.09)
	RIL 017 (10.71)	RIL 106 (10.97)	RIL 065 (10.67)
	RIL 139 (10.17)	RIL 119 (10.65)	RIL 135 (10.47)
	RIL 053 (10.06)	RIL 075 (10.59)	RIL 139 (10.14)
	RIL105 (9.86)	RIL 032 (10.48)	RIL 082 (9.97)

2.4 Conclusions

There have been relatively few reports on the popping quality of grain sorghum or potentially related kernel characteristics. This study evaluated the heritability of the two main popping traits valued by popcorn breeders in sorghum. Popping quality traits

in sorghum strongly are influenced heavily by genotype, environment and genotype x environment interactions. Even so, these traits are moderately to highly heritable within this population, even across three diverse environments within Texas.

While is it apparent that many traits influence popping characteristics, there is no single trait that that effectively predicts PE or ER in a given line. While some characteristics found to correlate with popping quality in popcorn correlate comparatively in sorghum others do not. Regardless, the results herein indicate that sorghum lines with improved popping quality can be developed via selective breeding. In fact, several of the RILs used for this study would serve as excellent candidates toward improvement of the trait. RIL065 is one such candidate as it had very high PE and ER when compared to the rest of the population and, in addition, had excellent stability as it performed well in all three environments.

3. QUANTITATIVE TRAIT LOCI FOR POPPING CHARACTERISTICS AND KERNEL CHARACTERISTICS IN SORGHUM GRAIN

3.1 Introduction

As one of the most drought-tolerant cereal grain crops, sorghum (Sorghum bicolor L Moench) is one of the staple food crops for millions of people in Africa and Asia. In other regions of the world, it is utilized primarily as a feed grain and forage with food grain as a niche market (Taylor 2006). In the US, it is becoming more common in human diets for several reasons. First, sorghum is high in antioxidant content and can be used as a functional ingredient in many foods (Dykes and Rooney 2006). Second, the absence of gluten in sorghum has increased its use in gluten-free products and diets (Taylor 2006). Finally, the absence of commercial GMO sorghums is appealing to those consumers who wish to avoid consumption of GMO products.

Another potential niche market for sorghum is as a popped grain, particularly when used in the production of confectionary foods. Modern breeding programs have been incredibly successful in improving popping traits in maize (*Zea mays*). While popcorn now has a 95% or better popping efficiency, or PE, and an excellent expansion ratio, or ER (Lyerly 1942; Pordesimo et al. 1991), very few studies have been conducted on popping quality in sorghum grown within the United States (Gaul and Rayas-Duarte 2008; Rooney and Rooney 2013). Rooney and Rooney (2013) demonstrated that it is possible to identify those genotypes of sorghum which have desirable popping characteristics.

There are multiple key factors that influence popping quality in sorghum grain. An understanding of the popping process itself is vital if these factors are to be put into perspective. Popping occurs when moisture in the center of the endosperm vaporizes and increases the pressure in the endosperm enough to rupture and burst the outer endosperm (Hoseney et al. 1983). The starch composition of the endosperm as well as the thickness of the pericarp are both integral to popping quality. Thicker endosperms, and thus greater kernel hardness, are most desirable for popcorn producers (da Silva 1993).

To improve popping quality in sorghum, a selective breeding approach similar to that used in popcorn is necessary. Breeding efficiency is increased when molecular tools are available; to that end, identifying QTL markers for PE and ER will improve the efficiency of a pop sorghum breeding program (Lu 2003; Collard 2005). QTL have been identified for popping quality traits and related traits in maize (Lu et al. 2003). Li et al. (2007) detected six QTL accounting for 54.0% of the total phenotypic variance in popping volume; five QTL accounting for 39.1% of the total phenotypic variance were found for popping rate. Lu et al. (2003) identified four QTL explaining 45.0% of the phenotypic variation for expansion volume in a popcorn x dent cross, analogous to expansion ratio in this study. Both of these studies demonstrate it is possible to find QTL for popping traits in corn and, thus, it is conceivable that a similar approach may be successful in sorghum.

Sorghum has a fully sequenced genome (Paterson et al. 2009), and sorghum improvement programs have benefitted greatly from advances in genotyping technology.

One of the newest and most effective techniques is that of digital genotyping. This technique can be used to quickly generate very accurate genotyping data which can then be used for a wide variety of analyses, including QTL mapping (Morishige et al. 2013). This DG technology was used in tandem with a standard QTL mapping methodology in order to identify regions of the genome that affect PE and ER in sorghum. Within this context, the objective of this study is to identify QTL for popping characteristics and kernel composition traits that might be associated with popping quality. Once identified, these QTL markers can be evaluated to determine if they enhance selection in a sorghum popping quality improvement program (Collard et al. 2005).

3.2 Materials and Methods

3.2.1 Experimental Design and Germplasm

A recombinant inbred line (RIL) population of 127 entries was derived from the cross of Sureño x RTx430 (Rodriguez-Herrera et al., 2000) and was the germplasm used for this study. Sureño has a pedigree of [(SC423 x CS3541) x E35-1]-2 and was released for its superb grain quality properties (Meckenstock et al., 1993). RTx430 has a pedigree of (Tx2536 x SC0170-6-5-1)-10-4-4-1-4 and was released based on its high levels of disease resistance and excellent general combining ability (Miller, 1984). Rooney and Rooney (2013) described differences in popping quality between derivatives of Sureño and Tx430; with Sureño derivatives popping very well and and RTx430 derivatives having poor popping characteristics. As such, RILs produced from a cross between these two parental lines were expected to segregate for popping quality traits. The RIL population was grown in multiple environments to produce grain for evaluation. The test was planted in a randomized complete block design (RCBD) layout

with two replications in three Texas locations; Weslaco, Corpus Christi, and Halfway. Plants grown in the Corpus Christi (CC) environment were grown in Orelia clay loam with the following rainfall: March = 3.43 cm, April = 6.55 cm, May = 7.49 cm, June = 3.91 cm, and July = 3.40 cm. Plants grown in the Halfway (HW) environment were grown in Pullman clay loam with the following rainfall: May = 2.69 cm, June = 8.53 cm, July = 1.22 cm, August = 2.18 cm). Plants grown in the Weslaco (WE) environment were grown in Hidalgo sandy clay with the following rainfall: February = 7.01 cm, March = 1.02 cm, April = 0.43 cm, May = 4.55 cm, June = 1.47 cm). Standard agricultural practices for sorghum grain were used in each location.

Grain was harvested just after black layer (physiological maturity) to minimize the effect of post-maturity grain weathering. A minimum of five panicles were harvested from each experimental unit; more if the size of the panicle was small to ensure sufficient quantities of grain for testing. These panicles were bulk threshed using a single head thresher (Alamaco) and to remove any remaining glumes or panicle residue the grain was then cleaned using a Wintersteiger LD180 (Wintersteiger Ag; Ried, Austria). Once threshed and cleaned, grain was packaged and stored at 10° C until further analyses.

3.2.2 Genotyping

The genotyping by sequencing approach, known as Digital Genotyping (DG), specifically developed for C4 grasses (Morishige et al., 2013) was used in this study. Seed of each recombinant line was germinated and grown for 14 days in Sunshine MVP growing media (Sun Gro Horticulture) in a greenhouse using sunlight as well as sodium

halide lighting. The FastPrep FP120 instrument (Bio 101, Savant) was used to extract total genomic DNA from the leaf tissue of each of the seedlings according to the manufacturer's protocol. Sequence-quality DNA was obtained via the FastDNA Spin Kit (MP Biomedicals). Purified genomic DNA was quantitated using a Qubit Fluorometer (Invitogrogen).

DG libraries were prepared for each sample using the restriction enzyme *Fse*I (New England Biolabs) according to the protocol described in Morishige et al. (2013). Briefly, 250 ng of each DNA was digested with *Fse*I and after digestion, 12 bp in-line barcodes were ligated to the fragments. Following ligation, all 127 progeny plus the two parental DNA samples were pooled. After randomly shearing the pool to a target size of 250 bp, it was size-selected on a 2% agarose gel for a size range of 250 +/- 50 bp. The size-selected sample was subjected to overhang fill-in, blunting and adenylation followed by ligation to an Illumina-specific adaptor and then purified using magnetic beads (Agencourt AMPure XP, Beckman Coulter). PCR was then performed on the pool using Phusion high-fidelity polymerase (Finnzymes). Dynabeads (Life Technologies) were used to obtain single-stranded products, and these products were amplified via PCR in order to incorporate the Illumina bridge amplification sequence. Following purification, products of the PCR were then quantified using PicoGreen fluorescent dye (Quant-iT dsDNA Broad Range [BR] kit, Life Technologies). The products of the PCR were then diluted to a concentration of 10 nM. The Agilent 2100 Bioanalyzer (Agilent Technologies) was used to assess the quality of the products. Sequencing of the template was performed on an Illumina HiSeq2500 (Illumina) using

standard Illumina protocols by Texas A&M AgriLife Genomic and Bioinformatics Services. Single-end sequencing was carried out for 125 bp.

FASTQ sequences were obtained from Texas A&M AgriLife Genomic and Bioinformatics Services and processed using a series of custom perl and python scripts. This process included the removal of sequences that did not contain the partial *Fse*I restriction site in addition to the 12 bp barcode identifier, the sorting of bar-coded sequences corresponding to each RIL or parent into separate files, and the compression of any duplicate reads. Removal of any sequences that matched more than one region of the Sbicolor_79 reference sorghum genome (www.phytozone.net/sorghum), accessed 2 Nov. 2014) was performed in order to prevent possible complications in the placement and order of markers in the genetic map. Polymorphisms between the two parents, RTx430 and Sureño, were identified and scored in the progeny lines as described by Morishige et al. (2013). JoinMap 4.0 software was utilized in order to construct a genetic map (van Ooijen and Voorrips, 2001).

3.2.3 Phenotyping of Material

Data was also collected for the following agronomic traits. Days to flowering was recorded as the number of days from planting to mid-anthesis of the panicle. Just prior to harvest, plant height was recorded as the distance from the tip of the panicle to the base of the plant. Grain from RIL population segregated for endosperm color (yellow to white), and this trait was measured using a subjective visual rating for grain color. These were taken as a numerical score, with 1 indicating a lighter, whiter sample and 9 indicating a darker, yellow endosperm. Kernel hardness was measured using a

Single Kernel Hardness Tester, model 4100 (Perten Instruments) where 300 individual kernels were crushed separately, calculating a mean and standard deviation. At the same time, measurements of individual kernel weight (mg) and diameter (mm) were recorded.

Popping quality for each entry was measured by estimating both popping efficiency (PE) and expansion ratio (ER). Because of the number of samples to pop in this study, methodology had to be developed. A set of modified heated air poppers (Presto 04821) was outfitted with steel wire mesh to keep the sorghum kernels from being blown out during the popping process. Each experimental unit was popped twice, in two different machines and each replication was blocked so that variation in poppers was partitioned into the replication effect. While optimum moisture content for popping in *Zea mays* is about 14-16% moisture (Gökmen 2004) and Rooney and Rooney (2013) adjusted moisture content of their samples to 15%, it was not feasible to adjust moisture content due to the sample numbers and size of the popped sample. Consequently, samples, which averaged 11% moisture with a range from 10-12% moisture content, were popped at their ambient moisture content.

For each sample, 500 seed were counted using an OLD MILL electronic seed counter Model 850-3. Prior to popping, seed volume (ml) of the sample was measured using a graduated cylinder and the weight of the sample was measured in grams. Each sample was placed in the popper which was turned on and run for 2 minutes and 15 seconds. This time duration was based on testing to identify optimum popping time. After popping, the sample was poured onto a 4.7 mm sieve and shaken. Any kernels that

fell through the sieve were considered un-popped, and any that remained above were considered popped. The volume of the popped fraction was measured in a graduated cylinder. The un-popped kernels were collected and recounted using the OLD MILL electronic seed counter Model 850-3.

Popping Efficiency is defined as the percentage of popped kernels divided by the total kernels popped (500) and it was calculated using the formula: $\frac{(500-UPK)}{500} = PE$ where UPK is the number of unpopped kernels remaining after popping. Expansion Ratio is the volume of the popped grain divided by the volume of the unpopped grain and was calculated using the original volume (mL) of 500 kernels (UPV) and the popped volume (mL). The formula for Expansion Ratio is $\frac{(PV)}{PE*UPV} = ER$.

3.2.4 QTL Analysis

A genetic map was constructed using Joinmap 4.0 software (van Ooijen and Voorrips 2001). Markers that were determined to be too close to one another (<10000 bp) were then assessed for their amount of missing data, and the markers with a significant amount of missing data were removed. Mapping was conducted using the Kosambi mapping function (Kosambi 1943). Phenotypic means for QTL analysis were calculated using Microsoft Excel software. Each of the phenotypic traits measured for the study was used to conduct composite interval mapping (CIM) QTL analysis. A total of 1000 permutations at 0.05 significance level were performed using Windows QTL Cartographer 2.5 software (Wang et al., 2012). The log of odds, or LOD, threshold was calculated using the method described by van Ooijen (1999). QTL were reported by

chromosome and environment, with chromosomes designated using the same methodology as in Kim et al. (2005).

3.3 Results and Discussion

3.3.1 Genetic Map

A dense map composed of 828 SNP distributed over all ten chromosomes was generated and used to conduct QTL analysis (Figure 1, Table 7). This map has a few large gaps (Figure 1) with the largest gap on chromosome 1 between 1.40 cM and 83.3 cM presumably due to a large amount of segregation distortion in that region (Figure 1). Other instances of segregation distortion are common in similar studies (Liu et al. 2010; Pereira et al. 1994). Other smaller gaps were found on chromosome 1 from 83.3 cM – 95.5 cM, chromosome 3 from 16.4 cM – 41.0 cM, chromosome 6 from 15.5 cM – 23.2 cM and 36.0 cM – 52.1 cM, chromosome 7 from 37.0 cM – 48.0 cM, and chromosome 8 from 4.5 cM – 29.8 cM. The overall average distance between markers was 2.0 cM across all chromosomes, while the total length of all of the mapped portions of the chromosomes combined was 1526.9 cM (Table 7). In Klein et al. (2001) which describes mapping this same population with AFLPs and

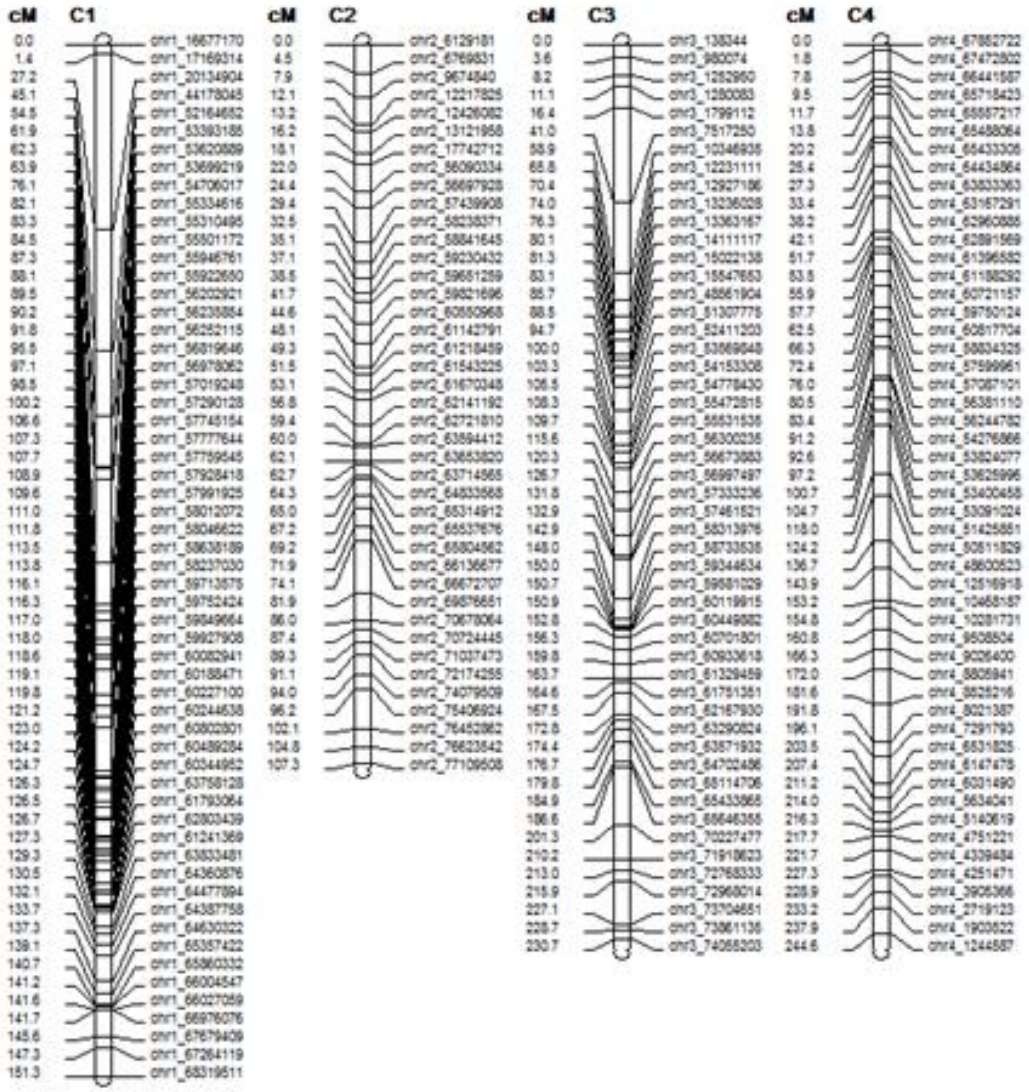

Figure 1: Genetic Linkage Map. Genetic linkage map. Genetic map was developed for a RIL population derived from a cross between R.Tx430 and Sureño. Chromosomes are arranged in order from left to right, with C1 = Chromosome 1, C2 = Chromosome 2, etc.

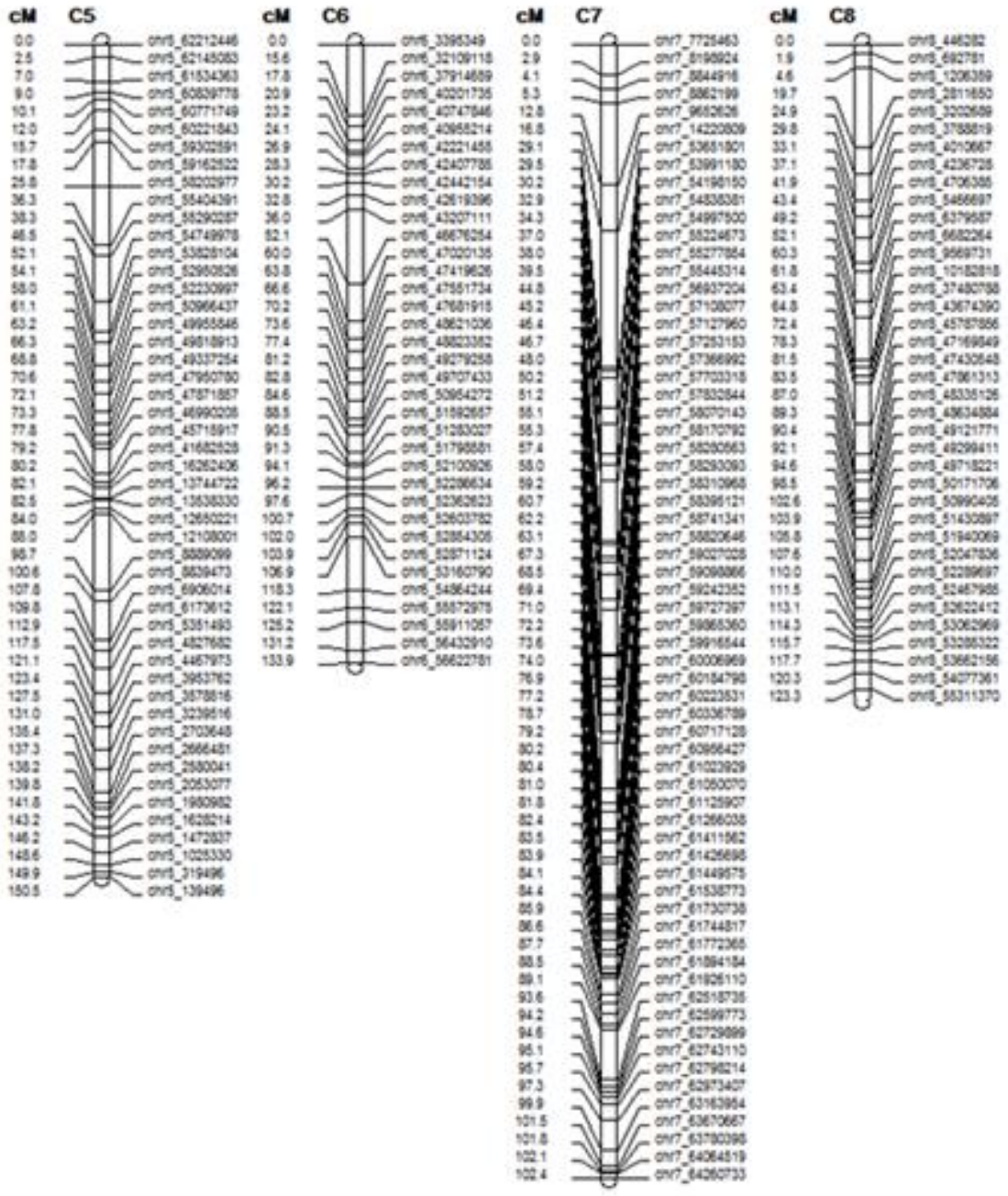

Figure 1 continued.

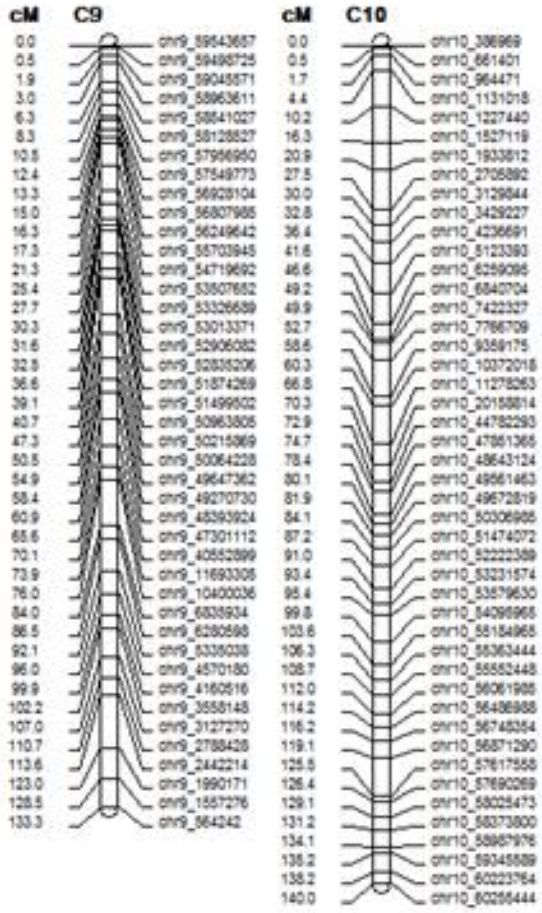

Figure 1 continued.

Table 7: Length of Coverage of the SNP Markers. Length of coverage of the SNP markers for each chromosome, the number of markers per chromosome, and the average distance between markers in the genetic linkage map of RIL from a cross of Sureño x RTx430.

Chromosome	Length	Marker number	Avg. distance between markers
	cM		cM
1	151.3	58	2.6
2	107.9	82	1.3
3	233.4	102	2.3
4	248.9	102	2.4
5	150.5	97	1.6
6	134.2	72	1.9
7	102.4	65	1.6
8	125.1	76	1.6
9	133.3	83	1.6
10	139.9	91	1.5
Total	1526.9	828	2.0

SSRs, the linkage map developed was composed of 130 markers on all ten chromosomes with a total length of 970 cM. The average distance between markers was 7.0 cM as opposed to 2.0 cM in the current study (Klein et al. 2001). Thus, not only did the map constructed for this study encompass a much larger area of the genome, it was also much denser and accounted for greater genetic recombination.

3.3.2 QTL for Agronomic Traits

For plant height, a total of five different QTL were detected for plant height across all three locations; three of these were consistently detected across all environments. (Table 8). Four QTL were found in CC, with two of these QTL having a large additive effect at 55.2 Mbp and 58.6 Mbp on chromosome 7 (Table 8). These QTL had high confidence at LOD scores of 14.25 and 16.18, respectively (Table 8). Four QTL were detected in the HW environment. Two of these, located at 55.2 and 58.6 Mbp on chromosome 7, had large effects of 4.1526 and -5.0621 respectively. (Table 8). Four QTL were identified in the WE environment for height; two of these were identified on chromosome 7 (Table 8). The additive effects of these two QTL were much higher in this location, with values of 8.9219 and -11.3103 and LOD scores of 14.95 and 23.01 (Table 8). For the majority of the QTL, increases in height were associated with the alleles from Sureño which is logical as Sureño is significantly taller than RTx430. However, the presence of taller alleles in RTx430 (which QTL) provides some explanation for the significant transgressive segregation that was reported in Chapter 2.

The height QTL on chromosome 7, which were detected across all environments, align with the known location of *Dw3* (Chittenden et al. 1994; Lin et al. 1995; Pereira and Lee 1995; Austin and Lee 1996; Peng et al. 1999). This population is segregating for the *Dw3* locus (Klein et al. 2001; Rodriguez et al. 1999). The high LOD score of these loci associated with *Dw3* confirms the accuracy of this genetic linkage map (Rodriguez et al. 1999; Burrell et al. 2014). Another QTL at 4.4 Mbp was detected in all three locations on Chromosome 9 (Table 8). This QTL was not identified in the prior

study by Klein et al. (2001); most likely because the current map has substantially greater marker density than the original map (Beavis et al. 1995; Klein et al. 2001).

Table 8: QTL Identified for Height and Flowering Date. QTL identified for height and flowering date within RILs derived from a cross between Sureño x RTx430. Environments include Corpus Christi (CC), Halfway (HW), and Weslaco (WE) in Texas. Positive additive effects are associated with the RTx430 allele while negative additive effects are associated with the Sureño allele.

Trait	Environment	Chromosome	QTL 1 LOD Interval (cM)	QTL Peak Position (cM)	QTL Peak Position (Mbp)	L.O.D.	R^2	Add. Effect
Plant Height	CC	4	84.5 - 91.3	90.5	54.3	3.62	0.051	-2.0181
		7	37.8 - 40.4	39.0	55.2	14.25	0.288	5.2963
		7	59.0 - 61.2	60.2	58.6	16.18	0.318	-5.6346
		9	93.3 - 99.9	99.0	4.4	9.83	0.156	-3.5812
	HW	7	32.8 - 42.6	37.1	55.2	8.81	0.194	4.1526
		7	60.5 - 61.9	61.7	58.6	11.54	0.288	-5.0621
		8	92.5 - 98.0	94.6	49.7	3.60	0.063	2.0855
		9	96.2 - 101.1	99.2	4.4	7.82	0.151	-3.2220
	WE	4	87.9 - 91.7	91.2	54.3	4.75	0.054	-3.5320
		7	36.1 - 37.5	37.1	55.2	14.95	0.277	8.9219
		7	61.1 - 61.8	61.7	58.6	23.01	0.435	-11.3103
		9	95.8 - 98.9	97.0	4.4	16.69	0.237	-7.5416
Flowering Date	CC	9	78.2 - 81.7	79.0	8.1	3.48	0.101	1.5867
	HW	4	213.2 - 217.3	216.3	51.0	4.88	0.126	1.3411
		8	94.6 - 98.6	97.3	50.1	3.72	0.096	1.1749
		9	39.0 - 40.7	39.7	50.9	3.38	0.090	1.1473
	WE	9	51.1 - 54.2	51.5	49.9	3.69	0.095	1.4196
		10	19.8 - 24.2	21.0	1.9	3.56	0.102	1.5333
Plant Color	All	6	122.8 - 124.2	123.1	~ 58.0	8.51	0.302	0.2697

Since *Dw1* is not segregating in this population, this height QTL on Chromosome 9 does not correspond to *Dw1*. In Brown et al. (2008), a QTL for plant height was found on chromosome 9, but it was in a completely different location the map used for that study. The identity of the QTL found here may be only significant within this particular genetic background though, notably, it isn't as strong as that found at the *Dw3* locus.

The effects of the height QTL were much stronger, comparatively, in the WE location (Table 8). Plants grown in the WE environment have delayed flowering times when compared to HW and CC and the increased time in vegetative growth could result in taller plants. This change in phenotype is thus reflected in the additive effect of the associated QTL (Table 8).

Plant color is a simply inherited trait and one QTL was detected for plant color at ~58.0 Mbp, or 123.1 cM on chromosome 6. This QTL had an additive effect of 0.2697 and a LOD score of 8.51 (Table 8). In Klein et al. (2001) this QTL was mapped to chromosome 6 at about 86.0 cM. The discrepancy in location between these two studies can likely be attributed to the difference in detection power in the two experiments, as stated in the case of plant height above (Beavis et al. 1995, Klein et al. 2001).

For flowering date, six QTL were identified in this population across all three environments (Table 8). One QTL was found in CC at 8.1 Mbp on chromosome 9, three QTL were found in the HW environment on chromosomes 4, 8, and 9, and two qtl were detected in WE on chromosomes 9 and 10 (Table 8). The QTL identified on chromosome 4 had both the highest LOD score and the highest additive effect. All of these QTL were unique to their respective environments; none of them were common

between environments. QTL for flowering date were not previously reported in Klein et al. (2001), but have been reported in numerous other studies in sorghum (Shiringani et al. 2010; Mannai et al. 2011; Reddy et al. 2013). In Shiringani et al. (2010) four QTL and one putative QTL were detected on chromosomes 3, 4, 6, 7 and 8. In Mannai et al. (2011), QTL for flowering time were found on chromosomes 1, 2, 3, 5, 6, and 10. Several of these chromosomes are in common with this study, though whether they are exactly the same can not be determined due to differences in the linkage map used.

3.3.3 QTL for Kernel Characteristics

For endosperm color, six QTL were identified (Table 9). In CC two QTL were identified on chromosome 7 at 59.2 Mbp and 61.9 Mbp (Table 9). A QTL at 59.1 Mbp was also found in HW and in both cases the allele contributing a yellow color was derived from RTx430 (Table 9). Three QTL were detected for the WE environment. One of these was located on chromosome 6 at 46.6 Mbp and had an effect of 0.5679 and a LOD score of 7.24 which was the largest of any endosperm color QTL in this study (Table 9). The other two QTL were on chromosomes 4 and 10 (Table 9). In all cases the alleles for yellow color were derived from Tx430. Previous studies have mapped QTL for endosperm color and carotenoid pigment content in sorghum but none of the QTL detected herein align with those reported in Fernandez et al. (2008). The lack of consistency could be due to a number of factors, including differences in genetic background, population size and map and marker density (Beavis, et al. 1993).

For kernel diameter, six different QTL were identified, with one of these QTL detected in all three environments (Table 9). In CC, three QTL were found: one on

chromosome 2, one on chromosome 3, and one on chromosome 9 (Table 9). Four QTL were found in the WE location on chromosomes 2, 8, and 9 (Table 9) and two QTL in HW on chromosomes 2 and 9. For the majority of these QTL, the allele that increases kernel diameter was contributed by RTx430. The common QTL was the QTL on chromosome 9 which was consistent in both genomic location (4.2 Mbp) and effect (larger kernels with the RTx430 allele) (Table 9).

Because there are no studies in sorghum for many of these traits, comparisons must be made with reports from other cereal grains. Choe and Rocheford (2011) detected QTL for kernel length (a similar measure of kernel size) were identified on chromosomes 1, 3, 6, 8, 9, and 10. In this QTL study, there were QTL identified on chromosomes 8 and 9, but they were not located in a similar point on the genome to the Choe and Rocheford (2011) study (Table 9). In Sun et al. (2009), numerous QTL were identified for both kernel length and kernel width, which are both functions of kernel size in wheat. QTL for length were identified on seven different chromosomes, but the locations changed depending on the environment with only the QTL on chromosome 4A being located in two environments. QTL for width, similarly, were found on three chromosomes with only one QTL at 6A being located in two environments. Those findings are similar to the ones found in this study, wherein many of the QTL for kernel diameter are not consistent across environments, with the exception of the one at 4.2 Mbp on chromosome 9 (Table 9).

For kernel weight, a total of ten QTL were identified with four QTL detected in CC and WE and two detected in HW, and all of these QTL located on chromosomes 1,

2, 5, 7, 8 and 9 (Table 9). The LOD scores for these QTL in all locations were comparatively high (Table 9). There was some consistency across locations for the precise location and effects of the QTL. For example, QTL on chromosome 7 were detected in CC and WE at similar genomic locations (58.4 Mbp) and effects (increased weight derived from Sureño) (Table 9). The QTL on chromosome 9 was also detected in CC. The QTL location in CC is a match for the location of the QTL for kernel diameter that was detected across all three environments, indicating that these traits may be influenced by some of the same loci or a series of linked loci (Table 9).

As far as is known, QTL analysis for kernel weight has not been reported in sorghum. In other grain crops, QTL for kernel weight have been reported. (Sun et al. 2009; Liu et al. 2014). In Liu et al., (2014) a large number of QTL were found for kernel weight in maize, sixteen in total, across chromosomes 1, 2, 3, 4, 5, 6, and 9. Comparatively fewer QTL were found for sorghum in this study, however, all of the QTL identified were located in at least two environments (Table 9).

Table 9: QTL Identified for Kernel Characteristics. QTL identified for kernel characteristics found to correlate with popping quality in sorghum grain. The population used for linkage mapping was a population of RILs in the F12 generation derived from a cross between Sureño x R.Tx430. Environments include Corpus Christi (CC), Halfway (HW), and Weslaco (WE) in Texas. Positive additive effects are associated with the R.Tx430 allele, while negative additive effects are associated with the Sureño allele.

Trait	Environment	Chromosome	QTL 1 LOD Interval (cM)	QTL Peak Position (cM)	QTL Peak Position (Mbp)	L.O.D.	R^2	Add. Effect
Endosperm Color	CC	7	68.4 - 70.6	69.4	59.2	6.69	0.165	0.7890
		7	88.6 - 91.3	89.1	61.9	4.06	0.093	-0.6024
	HW	7	67.1 - 71.1	69.4	59.1	4.18	0.114	0.6170
	WE	4	7.7 - 11.9	9.6	65.7	3.54	0.083	-0.3760
		6	52.2 - 59.1	54.2	46.6	7.24	0.182	0.5679
		10	84.8 - 86.7	85.6	51.3	6.65	0.169	0.5534
Kernel Diameter	CC	2	77.6 - 87.8	85.5	70.7	3.34	0.087	0.0466
		3	121.4 - 132.6	126.0	57.1	3.99	0.099	0.0490
		9	98.1 - 104.2	99.9	4.2	8.67	0.235	-0.0789
	HW	2	95.3 - 97.7	96.2	75.4	4.36	0.150	0.0490
		9	99.2 - 108.9	101.3	3.9	3.77	0.118	-0.0458
	WE	2	63.0 - 67.2	65.0	63.1	4.02	0.089	0.0501
		8	93.5 - 99.4	97.3	50.1	3.96	0.089	0.0492
		9	97.4 - 104.0	99.9	4.2	9.77	0.249	-0.0820
Kernel Weight	CC	5	140.7 - 143.0	141.8	1.8	3.44	0.089	0.9636
		7	33.5 - 36.4	34.3	54.9	6.24	0.185	1.6075
		7	60.0 - 61.8	60.7	58.4	7.39	0.220	-1.7181
		9	97.4 - 100.8	99.9	4.2	4.22	0.112	-1.0964
	HW	1	78.6 - 85.9	83.3	55.0	3.49	0.104	-1.0200
		8	100.3 - 105.7	102.6	50.9	3.45	0.101	1.0282
	WE	2	68.2 - 71.6	69.2	65.7	4.82	0.104	1.3018
		7	46.2 - 49.6	46.8	57.2	6.47	0.153	-1.9633
		7	58.4 - 61.9	60.7	58.4	7.62	0.174	-1.8888
		9	85.0 - 88.8	85.2	6.5	5.67	0.153	-1.7102
Kernel Hardness	HW	1	61.4 - 64.0	62.3	53.4	3.42	0.093	3.0359
		3	120.3 - 126.3	124.3	57.1	3.76	0.117	3.3912
		3	137.6 - 143.2	141.8	58.3	3.04	0.091	3.0088
		4	62.5 - 68.9	66.0	59.1	3.80	0.139	3.8042
		4	190.2 - 195.7	191.9	7.6	4.84	0.135	-3.6297
	WE	2	56.3 - 61.9	60.5	63.7	5.00	0.149	-4.1301
		4	0.0 - 7.5	2.8	66.8	4.16	0.128	3.8748
		4	7.7 - 16.0	11.7	65.6	4.62	0.128	3.8086

For kernel hardness, a total of eight QTL were identified with five QTL in HW and three in WE. No QTL for kernel hardness were detected in CC (Table 9). Pairs of QTL were found on chromosome 4 in both environments, but the genomic location was shifted (Table 9). A pair of QTL were also found on chromosome 3 in the HW environment, both with additive effects associated with Tx430 (Table 9). In the study conducted by Klein et al. (2001) which used the same population, two QTL for kernel hardness were identified at 25.0 cM on chromosome 4 and at 8.0 cM on chromosome 2. QTL in the same regions did not align (Table 9), but it is reasonable to assume some of this may be partially due to limitations of the earlier map or production environments (Beavis et al. 1995; Klein et al. 2001).

3.3.4 QTL for Popping Characteristics

For PE, a total of five QTL were identified across all three environments with one, two and two QTL detected in CC, HW and WE, respectively (Table 10). Favorable effects were derived from both the Tx430 and Sureño alleles (Table 10). The relative effect of these QTL was modest and there was no consistency in QTL detected across environments. For ER, four QTL were identified with one, two and one QTL detected in CC, HW and WE, respectively (Table 10). Favorable effects were all associated with the Sureño allele, which is consistent with the differences in ER between the parents (Table 10). The relative effect of these QTL was variable and, like PE, there was no consistency in QTL detected across environments for ER.

The inconsistency of these QTL across environments may be due to the relative importance of genotype, environment and genotype x environment interactions on

popping characteristics. Pugh et al. (Chapter 2) demonstrated that PE and ER are highly heritable but there is a significant environment and genotype x environment interaction. In maize, popping characteristics are also highly heritable but QTL for popping quality indicate a very complex trait with significant environmental interactions (Lu et al. 2003; Li et al. 2007; Yongbin et al. 2012). In Lu et al. (2002), QTL for popping expansion volume (ER) were detected on chromosomes 1, 3, 5 in maize, with the QTL on 5L being consistent across different populations and in different environments. Li et al. (2007), detected six QTL for popping volume (ER) and five QTL for popping rate (PE) on chromosomes 1, 2, 5, 6, 7, and 8, with the QTL on chromosome 5 corresponding to the one seen in Lu et al. (2002). In Yongbin et al. (2012), QTL for three different popping characteristics were found on chromosomes 1, 2, 4, 6, 7, and 10. These studies have identified QTL for the popping characteristics in different locations of the genome which supports the notion that popping is a very complex quality in maize, with many of the QTL being unique to their study with the exception of a QTL on chromosome 5 (Lu et al. 2002, Li et al. 2007). As seen in maize, the results of these studies suggest that popping in sorghum is a highly complex trait. None of the QTL found for either PE or ER overlapped with any of the QTL found for kernel endosperm, diameter, weight, or hardness (Table 9, Table 10). Pugh (Chapter 2) reported minimal correlations between these traits and either popping characteristic. This lack of association between these traits suggests that popping in sorghum is a highly complex trait that, while highly heritable, is controlled by many loci and is heavily contingent upon environmental factors.

Table 10: QTL Identified for Popping Quality Characteristics. QTL identified for popping characteristics. The population used for linkage mapping was a population of RILs in the F12 generation derived from a cross between Sureño x RTx430. Environments include Corpus Christi (CC), Halfway (HW), and Weslaco (WE) in Texas. Positive additive effects are associated with the RTx430 allele, while negative additive effects are associated with the Sureño allele.

Trait	Environment	Chromosome	QTL 1 LOD Interval (cM)	QTL Peak Position (cM)	QTL Peak Position (Mbp)	L.O.D.	R^2	Add. Effect
Popping Efficiency	CC	5	109.3 - 119.0	115.9	5.1	2.66	0.069	-0.0493
	HW	1	128.7 - 130.5	129.3	63.8	3.59	0.110	0.0444
		9	0.0 - 1.9	0.0	59.5	6.05	0.177	-0.0564
	WE	2	7.0 - 11.7	7.9	8.2	3.57	0.106	0.0580
		3	70.3 - 75.3	73.0	13.4	4.28	0.126	0.0845
Expansion Ratio	CC	3	85.5 - 95.0	91.5	52.7	3.49	0.109	-0.5538
	HW	5	66.1 - 68.4	67.0	49.7	5.23	0.153	-0.6390
		10	49.1 - 51.0	49.9	6.4	4.25	0.134	-0.5577
	WE	9	11.1 - 12.9	11.9	57.8	5.17	0.154	-0.7488

None of the QTL found for either PE or ER overlapped with any of the QTL found for kernel endosperm, diameter, weight, or hardness (Table 9, Table 70). Pugh (Chapter 2) reported minimal correlations between these traits and either popping characteristic. This lack of association between these traits suggests that popping in sorghum is a highly complex trait that, while highly heritable, is controlled by many loci and is heavily dependent on environmental factors just as in maize (Lu et al. 2003; Li et al. 2007; Yongbin et al. 2012; Pugh Chapter 2).

3.4 Conclusions

Herein are the first reports of QTL for kernel characteristics and popping characteristics in sorghum grain. QTL for PE and ER were environmentally specific and none were co-located with other kernel characteristics that have been associated with popping capacity in corn. This result indicates that popping in sorghum grain is a complex trait much like has been observed in corn (Lu et al. 2003; Li et al. 2007; Yongbin et al. 2012). However, multiple QTL with moderate to high additive effects were identified in this study indicating that selection to improve popping characteristics should be effective.

Of significant interest was the consistency of some of the QTL detected for kernel diameter and weight. The consistent detection of QTL for these traits implies that these loci are of significant importance in the genetic control of these traits. These QTL for kernel characteristics could be useful for improvement of other traits besides simply PE or ER and could have profound impact for breeders.

4. CONCLUSIONS

There have been relatively few reports on the popping quality of grain sorghum or potentially related kernel characteristics. This study evaluated the heritability of the two main popping traits valued by popcorn breeders in sorghum. Popping quality traits in sorghum strongly are influenced heavily by genotype, environment and genotype x environment interactions. Even so, these traits are moderately to highly heritable within this population, even across three diverse environments within Texas.

While is it apparent that many traits influence popping characteristics, there is no single trait that that effectively predicts PE or ER in a given line. While some characteristics found to correlate with popping quality in popcorn correlate comparatively in sorghum others do not. Regardless, the results herein indicate that sorghum lines with improved popping quality can be developed via selective breeding. In fact, several of the RILs used for this study would serve as excellent candidates toward improvement of the trait. RIL065 is one such candidate as it had very high PE and ER when compared to the rest of the population and, in addition, had excellent stability as it performed well in all three environments.

QTL for PE and ER were environmentally specific and none were co-located with other kernel characteristics that have been associated with popping capacity in corn. This result indicates that popping in sorghum grain is a complex trait much like has been observed in corn (Lu et al. 2003; Li et al. 2007; Yongbin et al. 2012). However,

multiple QTL with moderate to high additive effects were identified in this study indicating that selection to improve popping characteristics should be effective.

Of significant interest was the consistency of some of the QTL detected for kernel diameter and weight. The consistent detection of QTL for these traits implies that these loci are of significant importance in the genetic control of these traits. These QTL for kernel characteristics could be useful for improvement of other traits besides simply PE or ER and could have profound impact for breeders.

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