HIGH-DENSITY LINKAGE MAP CONSTRUCTION, MAPPING OF AGRONOMIC TRAITS IN TROPICAL MAIZE (ZEA MAYS L.) AND VALIDATING SNPS CONTROLLING MAIZE GRAIN YIELD AND PLANT HEIGHT IN SOUTHERN HYBRID TESTCROSSES

A Dissertation

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ABSTRACT

Texas, as the major maize producer in the Southern United States, faces serious problems in maize production (i.e. drought stress and aflatoxin contamination) as well as in maize breeding. In the Texas A&M maize breeding program, a previous genome-wide association study was conducted using a diverse panel of 346 inbred lines testcrossed to Tx714, and three quantitative trait variants (QTV1, QTV2 and QTV3) were identified, explaining 3-5% variation of grain yield under irrigated and non-irrigated conditions. In this present study, we constructed three bi-parental linkage populations (Ki3/NC356, Tx740/NC356 and LH82/LAMA-YC) and tested these as lines per se and as hybrid test crosses to validate three QTVs' effects and map QTLs for multiple agronomic traits using high-density SNP array.

The alleles at QTV1 and QTV3 from inbred line NC356 were detected significantly increasing plant height, flag leaf height and grain test weight in the Ki3/NC356 population across all tests over two years; QTV2 was identified significant with minor effects on flowering time in Ki3/NC356 $F_{3:4}$ progenies. In the other two populations, few consistent and significant QTVs' effects were validated, accounting for the limited population size and substantial field variation in our experimental environments.

Three high-density linkage maps were developed, with the average interval distance at 1.0cM. For the Ki3/NC356 population, a total of eighteen QTLs were detected for all traits using BLUEs; twenty two QTLs were detected when using BLUPs.

There were eight QTLs confirmed consistent by both BLUPs, and BLUEs and fourteen candidate genes were implicated. For the Tx740/NC356 population, twenty- five QTLs were detected using BLUEs and five consistent QTLs were identified by both BLUPs and BLUEs; six candidate genes were predicted. For the LH82/LAMA-YC population, only seven QTLs were mapped using BLUEs and one QTL was detected using BLUPs.

In this study, three bi-parental linkage populations were derived from tropical maize germplasm, which are adaptive to Texas environments and also good resources for Texas maize breeding. The QTLs identified in this study were promising candidates for further gene cloning and genic function analysis in future.

DEDICATION

To my parents, who gave me life, built the dreams for me and taught me to be a brave and independent person. Also to my boyfriend, Mr. Yaoyao Li, with your encouragements and love, I went through all the difficulties and frustrations during my PhD study in the United States.

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NOMENCLATURE

QTV	Quantitative Trait Variant
QTL	Quantitative Trait Locus
SNP	Single Nucleotide Polymorphism
RFLP	Restriction Fragment Length Polymorphism
SSR	Simple Sequence Repeat
CS	College Station
WE	Weslaco
PHT	Plant Height
FLH	Flag Leaf Height
EHT	Ear Height
DTA	Days to Anthesis
DTS	Days to Silk
GM	Grain Moisture
Tswt	Test Weight
GY	Grain Yield
CIMMTY	International Maize and Wheat Improvement Center
USDA-ARS	United States Department of Agriculture Agricultural Research Service

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction

The United States is the largest producer of corn in the world. The main production area is the temperate region of the Midwestern United States. Since the 1930s, the grain yield in maize has increased steadily through this region and most of the United States; but in the southern states yield has remained nearly flat (Barrero Farfan et al. 2013).

In the early part of the 20th century, George Harrison Shull first proposed that the corn production could be improved by 1) developing the inbred lines, 2) making crosses among the inbred lines to produce the hybrids, 3) evaluating and selecting the best hybrids in replicated trials and 4) reproducing the best hybrids seeds for farmers (Shull 1909).

This modern maize breeding involves two distinct activities: developing and improving inbred lines and hybrid development (and ultimately commercialization). Since the late 1930s, maize breeders have been continuously improving the grain yield by hybridizing two inbred lines. Heterotic groups and patterns are mostly fixed in the US and important to understand and maintain in hybrid breeding (Melchinger and Gumber 1998). As defined, heterosis (mid-parent) refers to the improvement between the hybrid

and the mean of its two parents. When the two inbred lines are crossed, vigor and grain yield of the hybrids exceeds the mean of the two parent lines. Assigning lines to heterotic groups improves heterosis and reduces the number of crosses that need to be investigated by crossing inbred lines from different heterotic groups; the hybrids performance greatly depends on the level of heterosis. In addition, creating the linkage mapping populations by crossing the parent lines belonging to different heterotic groups also allow more variation and polymorphisms in QTL analysis (Benchimol et al. 2000; Lai et al. 2010; Livini et al. 1992), however these findings are less relevant to breeding new lines.

In addition to being the second most cultivated crop in the world, maize (*Zea mays* L.) is also an important model plant for fundamental genetics and biology research. The maize genome is approximately 2,500 megabases, nearly 85% of which is made up of transposable elements (TEs) (Schnable et al. 2009). There are diverse resources relevant to maize genetics and biology studies, which are available to the public (http://maizegdb.org/). For example, MaizeCyc and CronCyc provide comprehensive metabolic pathway information; Corn Bin Maker (CBM) is a useful resource to explore the candidate genes in the particular bin of maize genome.

Most important agricultural trait variation in crops is complex and quantitative, such as flowering time, plant height, yield, etc. Usually these traits are controlled by many genetic loci with small effects. In order to uncover the genetic causative factors, to

date there are two most widely used approaches in plant quantitative traits studies: linkage mapping and association mapping.

Linkage Mapping Analysis

Traditionally, quantitative trait loci (QTL) linkage mapping provides insight into the genetic architecture of complex quantitative traits using linkage and generally relies on segregating populations derived from two parental inbred lines, like F_2, DHs (Double Haploids), BCs (Backcrosses), NILs (Near Isogenic Lines), RILs (Recombination Inbred Lines) and IBM (intermated B73× Mo17). Generally two steps are involved in QTL analysis: linkage map construction and QTL mapping. Two types of data are required for QTL analysis: phenotypic data and genotypic data.

A linkage map is a genetic map showing the relative position of the known genes or genetic markers in an experimental population. Rather than the specific physical map (determined from whole genome sequencing or cloning in vectors), a genetic map is based on the recombination frequencies between two genetic markers along homologous chromosomes during meiosis, which is an important prerequisite for QTL mapping. There are three general steps to create a linkage map: establishing the appropriate linkage groups by estimating the recombination frequencies of all pairs of markers across the whole genome; ordering the markers within a linkage group; and finally calculating the genetic distance between all pairwise markers (Broman 2010). The first

published maize linkage map just had 62 loci defined by morphological markers (Emerson, Beadle, and Fraser 1935). With the development of molecular markers, such as restriction fragment length polymorphisms (RFLPs), simple sequence repeats (SSRs), expressed sequenced tags (ESTs) and so on, a great number of QTL mapping studies have been performed in maize (http://maizegdb.org/). Since the advent of next generation sequencing, a large set of single nucleotide polymorphisms (SNPs) have been applied in high-throughput genotyping arrays (Ganal et al. 2011; Yan et al. 2010). In contrast to classical molecular markers, SNP genotyping arrays are more useful for high-density genetic mapping, which is expected to increase the mapping resolution and accuracy. More recently, a few high-density linkage mapping studies were reported in maize, improving the accuracy for detection of QTLs (Buckler et al. 2009; Guo et al. 2014; Pan et al. 2012; Peiffer et al. 2014; Raihan et al. 2016).

To date, there are three kinds of statistical models applied into QTL mapping: regression (Haley and Knott 1992; Whittaker, Thompson, and Visscher 1996), maximum-likelihood (Doerge, Zeng, and Weir 1997; Weller 1986) and Bayesian (Sillanpää et al. 1998). Based on different statistical model, many methods were proposed for QTL detection. The simplest approach is single marker analysis, which uses *t*-tests, analysis of variance (ANOVA) and linear regression. The main advantage of single marker analysis is that it does not require a linkage map and can be performed easily with basic statistical software, like JMP, SAS or R; however, this method is unable to determine the QTL position and probably underestimate the size of QTL effects due to recombination between markers and QTL (Collard et al. 2005). In order to

overcome such shortcomings, Lander and Botstein (1989) proposed the interval mapping (IM) method, searching for a putative QTL within an interval between two flanking markers. But it is easily to map the QTLs at wrong positions and with biased effects when more than one QTL are located on a chromosome when using the interval mapping method (Haley and Knott 1992; Martínez and Curnow 1992). To increase the precision and efficiency of QTL mapping, Zeng (1994) introduced the composite interval mapping (CIM) method, which combined interval mapping with multiple regression analysis. Recently a modified algorithm called inclusive composite interval mapping (ICIM) was proposed by Li, Ye, and Wang (2007), which keeps all merits of CIM with a faster convergence speed. It was reported ICIM reduced the rate of false detection and estimated QTL effects more precisely comparing to CIM and IM in bi-parental populations(Li et al. 2007).

Genome-wide Association Study

Due to the limited recombination events and low genetic diversity (at most two alleles per locus when using diploid inbred parents) between two parental lines of linkage population, linkage analysis has lower mapping resolution (QTL are generally localized at big genetic region on chromosomes) and only two allelic variants can be analyzed. In contrast, the genome-wide association study (GWAS) has been developed to examine the genome-wide associations between single nucleotide polymorphisms (SNPs) and phenotypes in a large set of distantly related individuals. GWAS is based on linkage disequilibrium and adjusts for relatedness and structure to reduce false positives

and maximize power (Mitchell-Olds 2010). The main advantages often noted for GWAS in diversity panels are high mapping resolution and simultaneous investigation of many alleles (S. Myles et al. 2009); less often mention is the ability to detect alleles across genetic backgrounds, minimizing genetic background epistasis (also known as context dependency). GWAS have detected many QTLs associated with agronomic traits in crops in the past decade (Cook et al. 2012; Farfan et al. 2015; X. Huang et al. 2010; Li et al. 2013; Peiffer et al. 2014; Tang et al. 2015; Tian et al. 2010; Warburton et al. 2015; Yano et al. 2016). However, it has been pointed out that strong population structure would induce spurious associations between phenotypic variations and unlinked markers (Lipka et al. 2015; S. Myles et al. 2009). Thornsberry et al., (2001) identified *Dwarf 8* (*d8*) was associated with flowering time in a diverse panel of 92 maize inbred lines. Recently Larsson et al., (2013) utilized more powerful statistical models and proved that *d8* associations were likely spurious associations in a more diverse panel of 282 inbred lines; and they suggested some traits (i.e. flowering time) were strongly correlated with population structure and the selection on these traits influenced the segregation patterns in the region. In order to address population structure problem in maize diversity association panels, new platforms are being used including NAM (Nested Association Mapping, McMullen et al. 2009; Yu et al. 2008) and MAGIC (Multi-parent Advanced Generation InterCrosses, Dell'Acqua et al., 2015; Holland et al., 2015) populations.

Texas, as the major maize producer in the Southern United States, faces the serious problems in maize production as well as in breeding. The temperate-adapted germplasm used by industry has impeded the maize production because it is not adapted

to the Southern US. Additionally, drought stress and alfatoxin contamination are the major constraints in Texas, but temperate germplasm is often poor for these problems (Betrán and Isakeit 2004; Betrán, Isakeit, and Odvody 2005; Mayfield et al. 2011). In order to improve the grain yield, breeding new varieties adapted to Texas growing conditions is of fundamental importance. Only a few genetic studies on maize have been conducted in Texas and only two of these have been relevant to using diverse germplasm to map QTVs (quantitative trait variants) and to make improvement in applied breeding (Farfan et al. 2015; Warburton et al. 2013).

In the Texas A&M maize breeding program, Farfan et al. (2015) used a diversity panel of 346 maize inbred lines testcrossed to Tx714 (Betrán et al. 2004) to evaluate the hybrids under irrigated and non-irrigated trials for grain yield, aflatoxin, plant height, ear height, flowering time and other important agronomic traits. Using 60,000 SNPs, they also conducted association mapping (GWAS) and identified 10 QTVs for grain yield, plant height, ear height and flowering time. Three of these QTVs (QTV1, QTV2 and QTV3) explained 5-10% variation of the grain yield under irrigated and non-irrigated condition (Table 1). Additionally, QTV2, which is located in bin 7.04, had a pleiotropic effect on plant height, days to anthesis and days to silk (data not shown).

Table 1. GWAS results for grain yield (ton/ha) remade from Farfan et al. (2015).

QTV	SNP	CHR	Effect(ton/ha)	R^2(%)	APE
CS11-WW-US					
QTV1	S2_27482479	2	0.26	3	C
QTV2	S7_164955163	7	0.37	4.9	C
QTV3	S9_142746374	9	0.28	3.6	G
CS12-WS-US					
QTV1	S2_27482479	2	0.31	3.6	C
QTV2	S7_164955163	7	0.42	5	C
QTV3	S9_142746374	9	0.33	3.9	G
CS12-WW-US					
QTV1	S2_27482479	2	0.28	3.6	C
QTV2	S7_164955163	7	0.14	4.9	C
QTV3	S9_142746374	9	0.28	3.5	G
MET analysis with spatial adjustment					
QTV1	S2_27482479	2	0.25	3.2	C
QTV2	S7_164955163	7	0.35	4.5	C

WW-water well; WS-water stress; R^2- percentage of variation explained by marker; APE- the allele of positive effect which increased yield.

QTL Analysis for Grain Yield

Grain yield is the most important trait in maize hybrid production and it is also one of the most complex quantitative traits in maize genetic studies. Due to the complex physiological processes and high sensitivity to environments, it is difficult to evaluate and improve the grain yield with its low heritability (Hallauer, A.R., M.J. Carena 2010). In the past decades, there have been many QTL mapping studies conducted for a variety of maize phenotypes. But there have been few significant common QTLs identified for controlling the grain yield across multiple environments (years and locations). Low heritability is a symptom of a high number of minor effect QTLs controlling maize grain yield, and these being very sensitive to interactions with the environment.

A series of QTL studies have previously reported QTLs related to maize grain yield in various linkage populations; it is important to note that these would be expected to be specific to the population and environments studied. A large QTL region on chromosome 5 was identified showing significant association with grain yield in a maize population generated from the cross B73×Mo17 by using restriction fragment length polymorphism (RFLP) molecular markers (Graham, Wolff, and Stuber 1997; Stuber et al. 1992). By comparing QTL detection for maize grain yield and yield components in $F_{2:3}$ and $F_{6:7}$ generations from the same population Mo17× H99, Austin and Lee (1996) identified six QTLs on five chromosomes accounting for 2.5% to 7.6% of the variation for grain yield, which collectively explained 21.8% of the phenotypic and 30.7% of the genotypic variation. By using 195 amplified fragment length polymorphism (AFLP) markers, a QTL mapping analysis was conducted in two sets of testcross progenies of 229 F_3 maize lines and identified several putative QTLs significantly affect grain yield on chromosome 1,2,4,6,7,9 and 10 by both simple interval mapping (SIM) and composite interval mapping (CIM) methods (Ajmone Marsan et al. 2001; Castiglioni et al. 1999). Y. Huang et al. (2010) used one conventional F_3 population and one advanced intermated F_3 population, both derived from the same parental maize inbred lines, to explore the genetic architecture of grain yield; totally, 9 additive QTLs detected for dry grain yield in the conventional F_3 population and 11 additive QTLs in the advanced intermated F_3 population. Sibov et al. (2003) mapped four QTLs for grain yield in a tropical maize population of 400 $F_{2:3}$ lines; these four QTLs located on chromosome 2,7 and 8 and two QTLs (*Gy2* and *Gy7*) overlapped with plant height QTLs (*Ph2* and *Ph7*)

separately, which was in agreement with observed traits correlation between grain yield and plant height. Another QTL mapping study in a tropical maize population of 256 $F_{2:3}$ families, Lima et al. (2006a) identified sixteen QTLs for grain yield, collectively explained 36.28% of the phenotypic variance; they also noticed that grain yield was positively correlated with plant height and ear height in the mapping population, as has been found important in Texas (Barrero Farfan et al. 2013).

QTL Analysis for Plant Height

Plant height is a key indicator of plant growth and can be an important contributor to crop yield. During the "Green Revolution", grain yields were increased significantly by introducing the dwarfing genes into many crops (Hedden 2003; Kush and Khush 2001). Particularly in maize breeding, the moderately short varieties are believed to have resulted in the higher planting density and great yield improvement in the U.S. Corn Belt (Johnson et al. 1985).

A number of dwarfing genes have been cloned in monocot crops and model organisms. The wheat mutant dwarfing alleles *Reduced height-1* (*Rht-B1 and Rht-D1*) encode proteins that participating in gibberellin signaling, which resulted in reducing response to gibberellin and plant height (Peng et al. 1999). In maize, the dwarfing gene *d8* and *d9* encode DELLA proteins, which repress GA-induced gene transcriptions in the absence of GA signaling (Fujioka et al. 1988; Harberd and Freeling 1989; Lawit et al. 2010; Winkler and Freeling 1994; Zentella et al. 2007). Arabidopsis Gibberellin Insensitive (GAI) gene and Slender Rice1 (SLR1) gene have been proved to be the

ortholog of *Rht and d8* (Ikeda et al. 2001; Peng et al. 1997, 1999). There are other genes that have been cloned in maize with the large effects on plant height. The *Dwarf3* (*D3*) gene of maize has significant sequence similarity to the cytochrome P450 gene, which encodes one of the early steps in Gibberellin biosynthesis (Winkler and Helentjaris 1995). In maize *brachytic2* (*br2*) mutants, the polar movement of auxins were hindered which resulted in compact lower stalk internodes (Multani 2003). Recently, one study mapped a major plant height QTL, *qph1*, to a 1.6kb interval in *Brachytic2* (*Br2*) coding sequence on maize chromosome 1. There was one rare SNP in *qph1*, which resulted in the impairment of the polar auxin transport in the mutant. In this study, the *pqh1* allele was validated to reduce the plant height significantly and also had a slight positively influence on yield (Xing et al. 2015).

However, some studies have found the positive correlation between maize grain yield with both plant height and ear height. Barrero Farfan et al. (2013) found that plant height, ear height, plant population and test weight were positively correlated with grain yield with stronger effects observed in the more stressed "rest of Texas" environments (which includes College Station) than in the Texas High Plains, and these were still stronger than typically observed in the Midwestern US. Others have reported that plant and ear height explained from 6% to 8% of the variation for maize grain yield under well-watered and well-fertilized conditions (Wiatrak and Liu 2011). Yin et al. (2011) reported that the corn yield was strongly related to plant height measurements made at the10-leaf (V10) and 12-leaf (V12) growth stages; they also concluded that corn yield could be predicted with plant height measurements during V10 to V12. These studies

evidenced that selecting taller plants would increase grain yield in particular environments.

As opposed to the low heritability of grain yield, maize plant height is much more heritable. Across NAM families in > 7 environments, the heritability of maize plant height has been estimated to be > 90% (Peiffer et al. 2014); in intermated B73×Mo17 (IBM, a Stiff Stalk × Non-Stiff Stalk cross) population, the plant height heritability was estimated to be 0.89 (Eichten et al. 2011). Many QTL analyses for maize plant height have been conducted in the past decades as the development of molecular markers in maize genomics has accelerated (Beavis et al. 1991; Bohn et al. 1996; Khairallah et al. 1998; Lübberstedt et al. 1997; Melchinger et al. 1998; Stuber et al. 1992; Veldboom and Lee 1994, 1996) (Table 2.). However, likely due to the different environments, various populations and limited molecular markers, there were no consensus QTLs identified.

More recently, with the exploration of maize genome as well as the development of various maize populations (such as near isogenic inbred, Inermated B73 × Mo17, etc.), a few QTL linkage mapping with high resolution revealed the genetic causative factors for maize plant height. Six QTLs were detected on chromosomes 5 (2 QTLs), 7, 8 (2 QTLs), and 9 by means of 193 pairs of simple sequence repeat (SSR) marker in a population (259 $F_{2:3}$ families) developed from a cross between a dent corn inbred and a popcorn inbred. Out of these 6 QTLs, there were 2 major QTLs (*qPH8-1* and *qPH8-2*) with contributions greater 22.6% and 19.3% respectively (Gustafson et al. 2007).

Eichten et al. (2011) used two sets of near isogenic lines (B73-like NILs and Mo17-like NILs), which were developed from the maize cross of two elite maize inbred lines B73 and Mo17, to detect the plant height QTLs; among the two sets of NILs, significant phenotypic variation ($p < 0.05$) was observed for plant height. In addition, one QTL located on chromosome 9 for plant height was commonly in the B73-like NILs, the Intermated B73 × Mo17 (IBM) population (Lee et al. 2002) and the North Carolina Recombinant Inbred (NCRI) population (Senior et al. 1996). Peiffer et al. (2014) fine mapped one QTL in two NILs possessing introgressions of the tropical lines CML277 and CML333 on the long arm of chromosome 9 in a B73 genetic background. This QTL interval was ~10Mb (CML277: 102,469,299- 109,910,100, CML333: 99,948,772-109,910,100, RefGenV1) in two sets of NILs, which contained more than 100 genes. The alleles from the two tropic lines CML277 and CML333 significantly increased plant height by ~ 5cm.

Unfortunately, many of the reported QTLs for maize height are buried in the literature and no thorough resource exists to compare them all. Of those that have been combined in a single resource, to date, over 219 QTLs for plant height that have been detected across the whole maize genome (data collected from Gramene QTL Database http://archive.gramene.org/qtl/).

Table 2. Summary of some previous QTL studies for plant height in maize.

Population	Population type	Population size	No. of QTL	Chromosome	Reference
B73 × Mo17	F_2	112	6	1,2,3,4,9,10	(Beavis et al. 1991)
B73 × G35	F_2	112	6	1,2,3,	(Beavis et al. 1991)
K05 × W65	F_2	144	3	5,8	(Beavis et al. 1991)
J40 × V94	F_2	144	3	6,7,9	(Beavis et al. 1991)
(B73/Mo17)-1-1-1)//B73	BCF_3	264	3	1,9,10	(Stuber et al. 1992)
(B73/Mo17)-1-1-1)//Mo17	BCF_3	264	5	2,3,4,7	(Stuber et al. 1992)
Mo17 × H99	$F_{2:3}$	150	5	1,2,4,6,7	(Veldboom, Lee, and Woodman 1994)
CML131 × CML67	F_2	171	4	2,3,4,5	(Bohn et al. 1996)
KW1265 × D146	F_3	380	30	1,2,3,4,5,6,7,8,9,10	(Lübberstedt et al. 1997)
Ki3 × CML139	$F_{2:3}$	472	11	1,2,3,4,5,6,7,8,9,10	(Khairallah et al. 1998)
KW1265 × D146	F_3	507	33	1,2,3,4,5,6,7,8,9,10	(Melchinger et al. 1998)

QTL Analysis for Flowering Time

Flowering time, as a highly heritable quantitative trait, is important for plants in the adaptation to environments and it also plays an important role in the vegetative to reproductive transition. Maize was domesticated between 6,000 and 10,000 years ago in southern Mexico, which is a typical tropical conditions-short days and warm temperature (Doebley 1990). Under the intense human selection, maize was cultivated to adapt to diverse environments and it now can grow in a wide range of latitudes all over the word. As an open-pollinated crop, asynchronous male and female flowering of maize would impact the grain yield, especially under drought conditions (Ribaut et al. 1996).

Multiple genes for flowering time and related traits have been positively identified. The maize *indeterminate 1 gene* (*id1*) located on chromosome 1 has been cloned, which encoded a zinc finger protein and controlled the transition of the shoot apex from vegetative to reproductive growth; *id1-m1* maize mutants produced more leaves and delayed flowering compared with the wild type (Colasanti, Yuan, and Sundaresan 1998). Across the maize genome, chromosome bin 8.05 has been a hot spot for flowering time; a few studies detected that QTLs for flowering time and the other correlated traits were at or near bin 8.05 (Chardon et al. 2005; Jiang et al. 1999; Phillips et al. 1992; Vladufu, McLaughlin, and Phillips 1999). Vladufu et al., (1999) identified two linked QTLs on bin 8.5, *Vegetative to generative transition 1* (*Vgt1*) and *2* (*Vgt2*) affected on days from sowing to pollen shed (DPS), plant height (PH) and plant node number (ND). Salvi et al. (2007) resolved the major flowering-time quantitative trait

locus, *Vgt1*, was located around 70kb upstream of an *Ap2-like* gene (*ZmRap2.7*); further study identified *ZmRap2.7* was orthologous to *Rap2.7* (also known as *TOE1*) in Arabidopsis, the function of which was a transcription factor regulating flowering time (Aukerman and Sakai 2003; Okamuro et al. 1997). Two duplicate *FLORICAULA/LEAFY* homologs *zfl1* and *zfl2* on chromosome 2 were reported to control inflorescence architecture and flower patterning in maize (Bomblies et al. 2003); and further study revealed that *zlf1*was more strongly associated with flowering time in maize (Bomblies and Doebley 2006). Another QTL meta-analysis for maize flowering time implicated that *zfl1* affected the variation of flowering time among various maize lines (Chardon et al. 2004). Previous QTL studies reported that the maize gene *Dwarf8* might affect flowering time (Koester, Sisco, and Stuber 1993; Schon et al. 1994); one association study also identified a suite of polymorphisms in gene *Dwarf8* associated with the variations of flowering time (Thornsberry et al. 2001). However since this study, *Dwarf8* has been shown to be a complicated locus with cryptic population structure which can lead to false positives in GWAS (Larsson et al. 2013). Taking advantage of the control over population structure and genetic background variation achieved by NAM population (McMullen et al. 2009; Yu et al. 2008), Buckler et al. (2009) investigated flowering time among 5,000 RILs and used multiple-family joint stepwise regression method to identify 36 and 39 QTLs that explained 89% of the phenotypic variance for days to anthesis and days to silking; and their results explained that the diverse flowering time among maize inbred lines were due to cumulative small-effect QTLs.

Photoperiod sensitivity complicates evaluation of flowering time and remains a key factor for maize flowering time regulation; tropical germplasm grown in middle latitudes is limited by extremely late flowering at temperate latitudes (Gouesnard et al. 2002). In temperate latitudes therefore photoperiod sensitive germplasm is perceived as extremely late flowering; while in tropical latitudes it may be average or even early. A major photoperiodic QTL in maize was mapped in the bin 10.04 region on chromosome 10 (Wang et al. 2008); later on, Hsiao-Yi Hung et al. (2012) identified the same QTL peak by genome-wide association analysis in NAM population, fine mapped QTLs on chromosome 10 in a maize-teosinte population and finally revealed *ZmCCT* as the most important photoperiod response gene in maize. Coles et al. (2010) identified four genetic regions controlling photoperiod response across four populations of RILs derived from crosses between two temperate inbred lines and two tropical inbred lines; these four regions were targeted at chromosome 1, 8, 9 and 10, which were referred as *ZmPR1-4* (for *Zea mays Photoperiodic Response*).

CHAPTER II

VALIDATING SNPS CONTROLLING MAIZE GRAIN YIELD AND PLANT HEIGHT IN SOUTHERN HYBIRD TESTCROSSES

Introduction

Maize is the primary feed grain in the United States, accounting for over 95 percent of total feed grain production. The countrywide maize production is forecast at a record 15.2 billion bushels with average yield at 175.1 bushels per acre (United States Department of Agriculture 2016). The top-producing areas concentrate in the Midwestern United States region known as Corn Belt. Texas, as the major maize producer in the Southern United States, has challenges in maize production as well as in breeding not experienced in the Corn Belt. The temperate-adapted germplasm used by the seed industry in the Corn Belt is not well-adapted to specific environments in Southern U.S., specifically, the major constraints are drought stress and alfatoxin contamination (Betrán and Isakeit 2004; Betrán et al. 2005; Mayfield et al. 2011). In order to improve grain yield, breeding new varieties adapted to Texas growing conditions is of fundamental importance.

Analyzing a historical dataset of Texas AgriLife Corn Performance Trials of commercial hybrids (2000-2010), Barrero Farfan et al. (2013) found that grain yield was positively correlated with plant and ear height, plant population, test weight and grain moisture and this correlation was more noticeable in the rest of Texas than in the high

plains (the northern and the western sides of Texas). This positive correlation between grain yield and plant height introduced a hypothesis that selecting a taller and non-lodging plant would improve maize grain yield, especially in stressed Texas environments.

Only a few genetic studies on maize have been conducted in the Southern US and even fewer in Texas; among these only two have investigated diverse germplasm to map QTVs (quantitative trait variants) for making improvements in applied breeding (Farfan et al. 2015; Warburton et al. 2013). Farfan et al., (2015) used a diversity panel of 346 maize inbred lines testcrossed to Tx714 (Betrán et al. 2004) to evaluate the hybrids under irrigated and non-irrigated trials for grain yield, aflatoxin, plant height, ear height, flowering time and other important agronomic traits. Using 60,000 SNPs, they conducted genome wide association mapping (GWAS) and identified 10 quantitative trait variants (QTVs) for grain yield, plant height, ear height and flowering time. Three of these QTVs (QTV1, QTV2 and QTV3) explained 3-5% variation of grain yield under irrigated and non-irrigated condition (Table 1). Among these, QTV2, located in bin 7.04, had a pleiotropic effect on plant height, days to anthesis and days to silk (data not shown). Because the diversity panel was grown as testcross hybrids, these QTVs have significant effects on hybrid phenotype, relevant to farmers, and act in a non-recessive manner with Tx714.

GWAS is a complementary tool to QTL linkage mapping, permitting the investigation of associations between single nucleotide polymorphisms and phenotypic

variances among a large number of unrelated individuals. The major advantages of GWAS are that it permits historical recombination events and multiple allelic variations to be investigated, which can result in a much higher mapping resolution (Sean Myles et al. 2009). Additionally, but less often mentioned that GWAS detects alleles across genetic backgrounds, minimizing the discovery of alleles affected by genetic background epistasis (also known as context dependency). There have been a number of GWAS reported and many QTLs associated with agronomic traits in maize (Andersen et al. 2005; Farfan et al. 2015; Larsson et al. 2013; Li et al. 2013; Peiffer et al. 2014; Thornsberry et al. 2001; Warburton et al. 2015; Weng et al. 2011). Although, strong population structure and relatedness were controlled in these studies, which could otherwise induce false positive results (Lipka et al. 2015; S. Myles et al. 2009), we are cautioned by the cryptic population structure of *dwarf8* (Larsson et al. 2013) and possibilities of overfitting the model. For a confirmation of GWAS detected loci, linkage mapping could be used in an independent bi-parental population, consisting of F_2 or recombinant inbred lines (RILs).

Near-isogenic lines (NILs) are often used to confirm the results of QTL mapping where progeny have already been derived from a bi-parental cross (Eichten et al. 2011; Koester et al. 1993; Mideros et al. 2014; Salvi et al. 2011; Szalma et al. 2007). The approach of NILs is less straightforward and requires more time to confirm GWAS results; which diversity panel lines should be crossed. It is often unknown which bi-parental crosses will detect the largest effect and minimize genetic background epistasis a priory, and developing multiple different donor recurrent parent NILs is expensive and

time consuming. In contrast, a bi-parental linkage population, built from these parents, can be screened at an earlier stage decreasing years of inbreeding and also allow improved inbred lines directly relevant to plant breeding can be simultaneously obtained. The limitations of a linkage population, primarily low resolution, are complementary to validating the high-resolution detection of GWAS. However, it is still possible that large effect in GWAS over diverse material may have context dependence and/or be masked by larger effect loci in any single bi-parental population. Therefore, it is important to use multiple populations in the validation of GWAS results. To date, there are few reported studies that have attempted to validate GWAS significant results in linkage populations, let alone using multiple populations.

The main objective of this study was to further validate GWAS results of three separate QTVs' effects on grain yield and other relevant agronomic traits in bi-parental linkage populations by single marker analysis and select the best performing lines for breeding.

Materials and Methods

Experimental populations

The three target QTV SNPs were first validated to segregate across eleven elite breeding lines by means of Sanger sequencing, as expected from the genotyping calls in previous GWAS (Farfan et al. 2015). These call were further confirmed using seven F_1 hybrids on-hand that were derived from these parents. The primers for Sanger sequencing were developed by Primer 3 (Untergasser et al. 2012), taking B73 maize

genome (Schnable et al. 2009) as reference; the primers information is provided in Table 3. All polymorphisms within the linkage populations were identified using ClustalX 2.1(Larkin et al. 2007). Three linkage populations from the initial seven F_1 hybrids were selected for further development and analysis because they had two or three of the previously detected QTVs confirmed as well as relevant from a breeding perspective to derive new elite Texas adapted inbred lines from. These three linkage populations were Ki3/NC356, Tx740/NC356 and LH82// (LAMA2002-12-1-B-B-B-B/LAMA2002-1-5-B-B-B-B)-3-2-B-1-B3-B (Table 4). (LAMA2002-12-1-B-B-B-B/LAMA2002-1-5-B-B-B-B) is a breeding line related to Tx740 (Mayfield et al. 2012), and will be referred to as LAMA-YC in this study.

Table 3. Primers for Sanger sequencing.

Orientation	Length	Tm	GC%	Sequence	Product size
FW-QTV1	20	57.5	40.0	CTGATCCATGAAAACGGATT	446
RV-QTV1	18	57.4	50.0	CGAGGATTTCCTGCTGAA	
FW-QTV2	20	57.6	50.0	ATGTACTCCCGATTGCTGAC	454
RV-QTV2	20	57.4	45.0	AGACAATTTCCCGCTCAGTA	
FW-QTV3	20	58.1	50.0	GTGTACTGCACAACGGATCA	430
RV-QTV3	20	58.0	45.0	GGATTTAGGCTGCAAGTGAA	

Table 4. Polymorphism of the three SNPs in the parent lines extracted from full sequences.

Pop.	Parental Lines	QTV1	QTV2	QTV3
1	Ki3	A	C	A
1&2	NC356	C	A	G
2	Tx740	A	C	G
3	LH82	C	A	G
3	LAMA-YC	A	C	G
	B73 (Ref.)	A	C	G

Population development, experiment design and phenotypic evaluation

Through selfing the $F_{2:3}$ progenies, $F_{3:4}$ individuals were produced in College Station, TX (CS14) in 2014 summer. Subsequently, $F_{3:4}$ progenies of each population were grown and evaluated at the winter nursery in Weslaco, TX, 2014 (WE14); each $F_{3:4}$ line was crossed to a Texas adapted inbred tester Tx714 to produce $F_{3:4}$ testcross hybrids, advanced to $F_{4:5}$ generation by selfing but were also measured for plant height. In the summer of 2015, $F_{3:4}$ testcross hybrid yield trials were evaluated for phenotype in both early (irrigated) and late (non-irrigated) planted trials in College Station, TX (CS15 yield trials) and as $F_{3:4}$ inbred trials.

Separately in the CS15 nursery, $F_{4:5}$ progenies were advanced to $F_{5:6}$ generation and testcrossed to Tx714 to produce $F_{4:5}$ testcross hybrids in the summer nursery. $F_{4:5}$ testcross hybrid yield trials were grown and evaluated for phenotype in Weslaco, TX, 2015.

College Station trials were located on Texas A&M University Farm in Burleson, TX on a ships clay loam soil. Weslaco trials were located in Texas A&M AgriLife Research and Extension Center at Weslaco, TX on a Hidago sandy clay soil. For the inbred and hybrid yield trials in College Station and Weslaso, each population was blocked separately and each experimental plot was 7.62 meters long and 76.2 centimeters wide. Depending on the available seed amount, the testcross hybrids and the commercial check line DK64-69 were laid out in the field for four replications, two replications or one replication (Table A1); each of $F_{3:4}$ inbred progeny were grown in two replications in inbred yield trials under irrigation. For the nursery, each experimental plot was 3.04 meters long and 76.2 centimeters wide; each entry replicate was grown as a single row plot due to seed limitations.

Days to silk (DTS, female flowering) and days to anthesis (DTA, male flowering) were estimated by 50% of the plants within each plot showing silks or shedding pollen. After pollination, one average-performing plant within each individual plot was selected for the measurements of plant height (PHT), flag leaf height (FLH) and ear height (EHT) in centimeter. Plant height was measured as the distance from the soil line to the top of the tassel; flag leaf height was measured from the soil line to the base of the flag leaf; ear height was measured from the soil line to the base of the top ear node. A HM-1000B Grain Gauge mounted on John Deere (Moline, IL) 3300 combine measured grain moisture (GM) was expressed as the percentage of the test weight; test weight (Tstwt) was determined as kg/hl. The grain yield value (GY) was calculated from

the plot weight and was standardized to 15.5% moisture and expressed as ton/hectare.

All the field tests and phenotypic measurements are summarized in Table 5.

Table 5. Number of plots and phenotypic measurements within each field test at College Station and Weslaco in 2014 and 2015.

	2014 winter	2015 summer		2015 winter
	Weslaco	College Station		Weslaco
Population	$F_{3:4}$ nursery	Yield trials on $F_{3:4}$ /Tx714 (irrigation / non-irrigation)	Phenotypic trials on $F_{3:4}$ (irrigation)	Yield trials on $F_{4:5}$/Tx714 (irrigation)
1	239	523 / 372	478	366
2	110	155 / 122	220	76
3	178	164 / 70	356	260
Planting date	Aug,8th 2014	Mar,2nd / Mar, 16th 2015	Mar, 9th 2015	Aug, 14th 2015
Phenotypic measurements	PHT, FLH, EHT	DTA, DTS, PHT, FLH, EHT, GM, Tstwt, GY	DTA, DTS, PHT, FLH, EHT	DTA, DTS, PHT, FLH, EHT

SNP genotyping

Total genomic DNA was extracted from a bulk of eight seedlings within each $F_{3:4}$ line (to capture segregation distortion and with the genotypes equivalent to the single progenitor F_3 plant) and the parental lines using modified CTAB method (Chen and Ronald 1999). To design the unique markers targeting the candidate QTVs, around 100 bp surrounding the three SNPs on either side were selected to pick the allele-specific primers and allele general primer using BatchPrimer3 v1.0(You et al. 2008). The primers sequences are presented in Table 6. KASP (Kompetitive Allele Specific PCR) assays (http://www.kbioscience.co.uk/) were used to conduct the genotyping for individual $F_{3:4}$ line.

Table 6. Primers information for KASP assays.

Orientation	Tm	GC%	Sequence
QTV1_F	56.8	50.0	CTCCTCCATATCCATCCAAC
*QTV1_R-C	57.3	47.6	GAAGGTCGGAGTCAACGGATTAAGCTCTGTGTCTTCTCATCG
ǂQTV1_R-T	57.3	45.5	GAAGGTGACCAAGTTCATGCTGAAGCTCTGTGTCTTCTCATCA
QTV2_F	57.9	50.0	GAGATGATGCAGCAGGAGAT
*QTV2_R-C	57.6	68.8	GAAGGTCGGAGTCAACGGATTGTCCTCCGCCTCCAAG
ǂQTV2_R-A	56.8	62.5	GAAGGTGACCAAGTTCATGCTGTCCTCCGCCTCCAAT
QTV3_F	59.1	41.7	GCAAGGAGAGCACCTAATTTATTC
*QTV3_R-G	60.7	48.0	GAAGGTCGGAGTCAACGGATTCTAAAGTTTGTAGAGGCAGCCTCTC
ǂQTV3_R-A	60.5	44.0	GAAGGTGACCAAGTTCATGCTCTAAAGTTTGTAGAGGCAGCCTCTT

*: allele specific primer with FAM-labelled tail; ǂ: allele specific primer with HEX-labelled tail.

Statistical analysis

Phenotypic data was analyzed using JMP® Pro 12.0.1(SAS Institute Inc., Cary, NC, 1989-2007.). A residual maximal likelihood (REML) approach was applied to conduct statistical analysis and single marker analysis. Multiple models were used to fit the data within and across tests. A random linear model (Eq. 1) was used to fit all the data within each test to estimate the variance components and factors explained no variations were excluded from the model. Best linear unbiased predictors (BLUPs) for each line in $F_{3:4}$ progenies and testcross hybrid ($F_{3:4}$/Tx714) were predicted for the following QTL mapping (in Chapter III).

Random model

$$Y_{ijklm} = \mu + G_m + T_i + C(T)_j + R(T)_k + r(T)_l + \varepsilon_{ijklm} \quad \text{(Eq. 1)}$$

where Y_{ijklm} is the trait value of each observation, μ is the grand mean; G_m is the random effect of each genotype m; T_i is random effect of each test i; $C(T)_j$ is random effect of range j nested in test i; $R(T)_k$ is the random effect of row k nested in test i; $r(T)_l$ is the random effect of replication l nested in test i; ε_{ijklm} is the random residual effect for each observation. Here rows were the lengths of the plots in the direction the tractor drove and irrigation was performed in the furrows between the rows. The ranges ran perpendicular to these rows.

Best Linear Unbiased Estimates (BLUEs) were also obtained for QTL mapping (in Chapter III) by modifying Eq. 1 to consider each genotype as a fixed effect.

Broad sense heritability on progeny-mean basis was calculated to determine how much of the phenotypic variation was attributed to genetic variance using (Eq. 2), in which σ_g^2 is the genetic variance expressed among the progeny in each population, σ_ε^2 is the residual error and r is the average number of replications for each progeny.

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + {}^{\sigma_\varepsilon^2}/_r} \quad \text{(Eq. 2)}$$

Validating QTVs' effects by ANOVA analysis

A multiple regression model (Eq. 3) was used to validate QTVs' effects in each linkage population. In this model, each marker genotype was fitted as a fixed effect and the other factors were fitted as random effects; the non-significant markers were dropped out of the model in the final analysis.

Mixed model

$$Y_{ijklm} = \mu + QTV_1 + QTV_2 + QTV_3 + T_i + C(T)_j + R(T)_k + r(T)_l + \varepsilon_{ijklm} \quad \text{(Eq. 3)}$$

where Y_{ijklm} is the trait value of each observation, μ is the grand mean; QTV_m is the fixed effect of each SNP m; T_i is random effect of each test i; $C(T)_j$ is random effect of range j nested in test i; $R(T)_k$ is the random effect of row k nested in test i; $r(T)_l$ is the random effect of replication l nested in test i; ε_{ijklm} is the random residual effect for each observation.

For each QTV validated in the mixed model, the additive effect was estimated as $a = (\mu_{BB} - \mu_{AA})/2$ and the dominance effect $d = \mu_{AB} - (\mu_{AA} + \mu_{BB})/2$; in which μ_{AA} and μ_{BB}

were the phenotypic means of parental lines and μ_{AB} was the phenotypic mean values of heterozygotes in $F_{3:4}$ progenies.

Results and Discussion

Phenotypic analysis

Phenotypic measurements were recorded for each population in Texas (College Station and Weslaco) over two years, 2014 and 2015, as illustrated previously in Table 4 (Materials and Methods). The environments othe tests grown in College Station, 2015 were characterized by sufficient rainfall and in one instance submergence of part of the field for multiple days before flowering. This weather pattern nearly eliminated visual distinctions between irrigated and non-irrigated tests.

Across all three populations grown as $F_{3:4}$ inbred phenotypic trials (College Station, 2015), the populations demonstrated transgressive segregation for all traits except days to flowering in population LH82/LAMA-YC (Table 7). For most traits, the population means exceeded either parent, but this was likely due to residual heterozygosity in $F_{3:4}$ progeny lines per se.

For $F_{3:4}$/Tx714 hybrid yield trials in College Station, 2015, the entry mean values of most traits across all three populations exceeded the check line DK64-69 under both growing conditions (non-irrigated and irrigated), particularly plant height and grain yield. It also was observed that the grain moisture means for $F_{3:4}$/Tx714 hybrid populations were more than DK64-69 across all tests, likely due to later maturity and

tighter husk coverage. After adjusting grain yield for moisture it was evidenced that the three populations have the potential to improve grain yield in Texas over an elite commercial hybrid (Table 8).

Rain in May and June, 2015, caused the Brazos River to overflow and the yield trials were submerged for multiple days. The irrigated tests were therefore not irrigated as initially designed. This left the main important difference between non-irrigated tests and irrigated tests for each population was planting date; the irrigated tests were planted in the early of March (March 2nd, 2015), and the non-irrigated tests were planted on March 16th, 2015. Constraints on available seed and limited field size, resulted in different number of entries in the two irrigation treatments (Table A1). For example, in the Ki3/NC356 population, there were 101 entries (CS15YKW21 and CS15YKW11) just tested in the early-planted irrigated field. Due to these weather conditions in the summer of 2015, the grain yield means in the irrigated tests for each population exceeded the non-irrigated tests (Table 8). This indicated the different planting date affected the grain yield and some entries in each population have high yield potential.

Compared to $F_{3:4}$/Tx714 hybrid yield trials in College Station, 2015, $F_{4:5}$/Tx714 hybrid and check line DK64-69 flowered around 20 days earlier in the 2015 winter Weslaco trial (Table 9).

Table 7. Summary statistics of parental lines and $F_{3:4}$ progenies for each trait by each population.

	Weslaco, 2014			College Station, 2015				
	$F_{3:4}$ inbred nursery			Ki3	NC356	$F_{3:4}$ inbred trials		
Trait	Min.	Max.	Mean ± S. D.	Mean	Mean	Min.	Max.	Mean ± S. D.
DTA (days)	na	na	na	88.5	87.5	77.0	92.0	85.4 ± 2.5
DTS (days)	na	na	na	89.5	90.0	79.0	94.0	86.1 ± 2.7
PHT (cm)	109.2	198.1	154.6 ± 15.4	124.5	147.3	109.2	190.5	149.5 ± 16.9
FLH (cm)	81.3	162.6	121.9 ± 14.7	96.5	113.0	78.7	157.5	117.3 ± 15.6
EHT (cm)	25.4	81.3	53.1 ± 11.4	33.0	52.1	20.3	81.3	51.4 ± 11.3
	Weslaco, 2014			College Station, 2015				
	$F_{3:4}$ inbred nursery			Tx740	NC356	$F_{3:4}$ inbred trials		
Trait	Min.	Max.	Mean ± S. D.	Mean	Mean	Min.	Max.	Mean ± S. D.
DTA (days)	na	na	na	92	88.5	84.0	92.0	88.4 ±1.8
DTS (days)	na	na	na	98.5	96.5	83.0	103.0	94.3±4.2
PHT (cm)	147.3	210.8	179.6 ± 12.2	123.2	113	101.6	167.6	131.2 ±14.6
FLH (cm)	86.4	152.4	139.8 ± 11.9	95.3	82.6	73.7	132.1	98.9 ±13.3
EHT (cm)	22.9	71.1	64.2 ± 10.1	39.4	35.6	22.9	66.0	44.8 ±9.2
	Weslaco, 2014			College Station,2015				
	$F_{3:4}$ inbred nursery			LH82	LAMA-YC	$F_{3:4}$ inbred trials		
Trait	Min.	Max.	Mean ± S. D.	Mean	Mean	Min.	Max.	Mean ± S. D.
DTA (days)	na	na	na	78.0	93.0	84.0	87.0	85.3 ± 0.7
DTS (days)	na	na	na	80.5	98.0	85.0	88.0	86.8 ± 0.6
PHT (cm)	119.4	195.6	151.6 ± 15.7	88.9	119.4	76.2	154.9	114.0 ± 14.0
FLH (cm)	86.4	152.4	117.4 ± 13.5	64.8	95.3	53.3	116.8	85.3 ± 11.4
EHT (cm)	22.9	71.1	47.5 ± 9.5	21.6	29.2	12.7	50.8	30.5 ± 7.4

Mean: arithmetic means; S.D.: standard deviation; Min.: minimum value; Max.: maximum value.
na: non-available.

Table 8. Summary statistics of $F_{3:4}$/Tx714 hybrids and check line DK64-69 for each trait by each population across all trials in College Station, 2015.

	Hybrid yield trials under non-irrigation, College Station, 2015				Hybrid yield trials under irrigation, College Station, 2015			
	$F_{3:4}$/Tx714 hybrid			DK64-69	$F_{3:4}$/Tx714 hybrid			DK64-69
	Ki3/NC356							
Trait	Min.	Max.	Mean ± S. D.	Mean	Min.	Max.	Mean ± S. D.	Mean
DTA (days)	65	79	71.1 ± 2.9	68.8	74	82	78.3 ± 1.6	77.5
DTS (days)	67	82	73.5 ± 3.1	71.3	76	86	80.7 ± 1.8	79.0
PHT (cm)	193	269.2	236.5 ± 17.9	216.9	198.1	246.4	223.5 ± 10.5	215.1
FLH (cm)	139.7	231.1	189.9 ± 18.9	178.3	154.9	213.4	182.8 ± 10.8	168.4
EHT (cm)	58.4	134.6	99.0 ± 16.4	83.3	63.5	124.5	94.1 ± 10.8	78.3
GM (%)	11.75	12.45	12.12 ± 0.13	12.5	10.26	16.46	13.21 ± 1.20	14.39
Tstwt (kg/hL)	54.32	61.32	57.80 ± 1.30	54.72	55.23	62.9	59.07 ± 1.42	56.34
GY (ton/ha)	3.5	11.1	7.5 ±1.6	7.1	3.1	14.1	8.9 ± 2.1	6.9
	Tx740/NC356							
Trait	Min.	Max.	Mean ± S. D.	Mean	Min.	Max.	Mean ± S. D.	Mean
DTA (days)	65	71	68.1 ± 1.1	65	78	81	79.23 ±0.85	79.4
DTS (days)	68	76	71.7 ± 1.3	68	79	86	81.98 ±1.32	82.6
PHT (cm)	236.2	279.4	258.2 ± 9.3	na	213.4	256.5	234.81 ±8.77	226.1
FLH (cm)	190.5	231.1	209.7 ± 9.0	na	170.2	210.8	190.67 ±8.27	168.6
EHT (cm)	86.4	139.7	110.6 ± 9.6	na	76.2	124.5	99.57 ±9.81	85.3
GM (%)	11.73	12.7	12.19 ± 0.19	12.35	10.37	15.37	12.92 ±1.08	15.60

Table 8. Continued

	Hybrid yield trials under non-irrigation, College Station, 2015				Hybrid yield trials under irrigation, College Station, 2015			
	$F_{3:4}$/Tx714 hybrid			DK64-69	$F_{3:4}$/Tx714 hybrid			DK64-69
Tstwt (kg/hL)	54.46	60.83	57.56 ± 1.29	54.18	55.04	61.65	58.74 ±1.37	56.88
GY (ton/ha)	4.8	10.2	7.6 ± 1.2	6.5	7.63	13.91	10.79 ±1.24	11.4
LH82/LAMA-YC								
Trait	Min.	Max.	Mean ± S. D.	Mean	Min.	Max.	Mean ± S. D.	Mean
DTA (days)	65	69	66.7 ± 1.3	66.0	74	77	75.7 ± 0.9	75.8
DTS (days)	67	72	69.5 ± 1.4	69.0	77	80	78.4 ± 0.8	78.1
PHT (cm)	215.9	264.2	235.7 ± 12.1	219.7	185.4	241.3	214.1 ± 10.8	198.4
FLH (cm)	170.2	218.4	192.1 ± 11.9	176.5	149.9	198.1	174.3 ± 9.7	166.1
EHT (cm)	76.2	127	100.3 ± 10.8	87.6	68.6	106.7	88.8 ± 8.4	82.7
GM (%)	11.62	13.03	12.32 ± 0.27	12.29	9.47	14.56	11.82 ± 1.15	13.51
Tstwt (kg/hL)	54.38	59.93	57.02 ± 1.36	53.84	54.32	61.8	58.03 ± 1.41	56.76
GY (ton/ha)	4.6	10.7	8.0 ± 1.5	6.0	5.4	13	9.3 ± 1.7	9.5

Mean: arithmetic means; S.D.: standard deviation; Min.: minimum value; Max.: maximum value.
na: non-available.

Table 9. Summary statistics of $F_{4:5}$/ Tx714 hybrid and check line DK64-69 for each trait by each population in Weslaco, 2015.

Population	Ki3/NC356			Tx740/NC356			LH82LAMA			DK64-69 (BLUP)		
Trait	Min.	Max.	Mean ± S. D.	Min.	Max.	Mean ± S. D.	Min.	Max.	Mean ± S. D.	Min.	Max.	Mean ± S. D.
DTA (days)	51.0	54.0	52.6 ± 1.0	52.0	55.0	53.5 ± 0.8	48.0	53.0	50.9 ± 1.0	50.9	53.5	52.3 ± 1.2
DTS (days)	51.0	54.0	52.4 ± 1.0	52.0	55.0	53.5 ± 0.8	50.0	53.0	51.4 ± 0.8	51.3	52.4	51.8 ± 0.6
PHT (cm)	218.4	279.4	249.8 ± 12.6	205.7	261.6	235.6 ± 12.7	195.6	271.8	231.7 ± 14.0	218.0	241.6	229.9 ± 10.5
FLH (cm)	170.2	231.1	202.1 ± 12.5	162.6	205.7	186.8 ± 10.6	157.5	218.4	188.2 ± 11.5	174.2	191.1	183.5 ± 7.6
EHT (cm)	71.1	139.7	105.8 ± 14.0	58.4	119.4	88.7 ± 13.3	55.9	124.5	89.9 ± 12.9	76.3	93.9	86.0 ± 8.0

Mean: arithmetic means; S.D.: standard deviation; Min.: minimum value; Max.: maximum value.
na: non-available.

Variance components estimates and heritability analysis

A linear random model (Eq. 1) was performed to partition the phenotypic variation into genetic variation (σ_g^2) and other sources of variations (σ_T^2, σ_C^2, σ_R^2, and σ_r^2). Broad-sense heritability on progeny-mean basis was calculated using Eq. 3. The percentage of total variance explained by each component and heritability on progeny-mean basis for each trait can be found in Table 10 -Table 12.

In $F_{3:4}$ inbred trials of the population Ki3/NC356, genetic variations for flowering time (DTA and DTS) explained a large amount of the total variations. For plant height, flag leaf height and ear height, the variations from replication, row and random error totally accounted for over 50% of the total variations. One likely reason for this random error was that while flowering time notes were recorded by one person, plant height measurements were taken by multiple people. Another factor for higher random error was that the inbred trials suffered from flooding, which resulted in high rows and replication variances in the field.

In $F_{3:4}$ inbred trials of the population Tx740/NC356, the genetic variation for days to anthesis and ear height were relative high, 54.86% and 41.52% respectively; for the other traits, the variations from ranges, rows and random error were much more than genetic variation.

The population LH82/LAMA-YC happened to blocked in the inbred trials field, where flood damage was worst, random error was the most obvious for all traits. Most

variation of flowering time (DTA and DTS) was unexplained random error and heritability of these two traits were very low (Table 12). A primary reason was that LH82/LAMA-YC $F_{3:4}$ progenies were laid out in the flooded part of the field; however, this is insufficient to explain the odd progeny regression to the mean for flowering time because the parent lines did not show this pattern. Because LH82 was a temperate ex-PVE line and LAMA-YC was derived from tropical germplasm, there were many morphological differences between these two parental lines and their $F_{3:4}$ progenies; but there was few differences among these progenies for flowering time (Table 7). This suggested the infinitesimal model where many infinite number of unlinked loci with an infinitesimal effects are controlling flowering time differences between the parents and in the population LH82/LAMA-YC.

In testcross hybrid yield trials over two consecutive seasons (CS15 and WF15) for all three populations, more unexplained error variation than typical was observed across traits, almost exclusively due to excessive rainfall (Tables 10, 11& 12).

According, broad sense heritability estimates on progeny-mean basis for each trait ranged from 0.03 to 0.86 across all trials in the three populations with grain yield heritability much lower than other traits. The heritability of most traits in the $F_{3:4}$ inbred trials were higher than those estimated in testcross hybrid trials, which accounted for few genetic variation in testcross hybrid trials.

Table 10. The percentage of total variance explained by each variance component and heritability on progeny-mean basis for each trait across all trials in population Ki3/NC356.

Trait	DTA (days)	DTS (days)	PHT (cm)	FLH (cm)	EHT (cm)	GM (%)	Tstwt (kg/hL)	GY (ton/ha)
$F_{3:4}$ inbred trials (College Station, 2015)								
Pedigree (σ_g^2)	57.6	48.8	37.5	31.0	22.2	na	na	na
Range (σ_C^2)	4.1	0.5	6.3	2.5	1.6	na	na	na
Row (σ_R^2)	4.5	4.9	12.6	14.0	6.3	na	na	na
Rep (σ_r^2)	14.3	18.4	23.5	32.3	35.5	na	na	na
Residual (σ_ε^2)	19.5	27.4	20.1	20.2	34.5	na	na	na
H^2	0.9	0.8	0.8	0.8	0.6	na	na	na
$F_{3:4}$/ Tx714 hybrid yield trials under non-irrigation (College Station, 2015)								
Pedigree (σ_g^2)	25.9	18.2	14.1	16.3	11.3	32.0	59.4	3.3
Range[Test] (σ_C^2)	2.8	1.7	44.3	58.9	51.2	-	2.7	1.9
Row[Test] (σ_R^2)	5.2	4.3	1.0	1.8	-	-	12.3	15.7
Rep[Test] (σ_r^2)	16.8	26.8	2.9	4.6	-	-	-	45.6
Test (σ_T^2)	20.1	24.4	27.0	-	15.4	-	-	3.6
Residual (σ_ε^2)	29.2	24.6	10.8	18.4	22.1	68.0	25.7	29.9
H^2	0.7	0.7	0.8	0.7	0.6	0.6	0.9	0.3
$F_{3:4}$/ Tx714 hybrid yield trials under irrigation (College Station, 2015)								
Pedigree (σ_g^2)	8.4	12.0	16.7	19.4	21.1	40.7	36.4	8.1
Range[Test] (σ_C^2)	5.2	3.4	3.8	3.7	1.5	-	-	4.4
Row[Test] (σ_R^2)	10.1	12.6	10.5	7.5	4.6	17.3	7.4	10.5
Rep[Test] (σ_r^2)	3.3	4.0	19.7	16.8	10.5	-	0.4	9.7
Test (σ_T^2)	10.2	13.1	-	-	-	-	-	38.3
Residual (σ_ε^2)	62.9	55.1	49.3	52.7	62.3	42.1	55.9	28.9
H^2	0.3	0.4	0.5	0.5	0.5	0.7	0.6	0.4

Table 10. Continued

Trait	DTA (days)	DTS (days)	PHT (cm)	FLH (cm)	EHT (cm)	GM (%)	Tstwt (kg/hL)	GY (ton/ha)
$F_{4:5}$/Tx714 hybrid yield trials (Weslaco, 2015)								
Pedigree (σ_g^2)	11.6	4.7	19.6	22.1	17.3	na	na	na
Range (σ_C^2)	17.9	9.1	23.7	12.5	4.4	na	na	na
Row (σ_R^2)	5.2	-	7.5	6.9	7.0	na	na	na
Rep (σ_r^2)	4.4	16.7	-	4.7	11.8	na	na	na
Residual (σ_ε^2)	60.9	69.5	49.1	53.8	59.6	na	na	na
H^2	0.3	0.1	0.4	0.5	0.4	na	na	na

na:non-available data; "-" represents the factor was removed from the model.

Table 11. The percentage of total variance explained by each variance component and heritability on progeny-mean basis for each trait across all trials in population Tx740/NC356.

Trait	DTA (days)	DTS (days)	PHT (cm)	FLH (cm)	EHT (cm)	GM (%)	Tstwt (kg/hL)	GY (ton/ha)
$F_{3:4}$ inbred trials (College Station, 2015)								
Pedigree (σ_g^2)	54.9	21.4	29.3	29.9	41.5	na	na	na
Range (σ_C^2)	6.9	37.2	20.1	23.8	15.6	na	na	na
Row (σ_R^2)	12.1	2.1	20.9	13.5	13.2	na	na	na
Rep (σ_r^2)	-	16.9	1.7	4.4	-	na	na	na
Residual (σ_ε^2)	26.2	22.5	28.1	28.5	29.7	na	na	na
H^2	0.8	0.7	0.7	0.7	0.7	na	na	na
$F_{3:4}$/ Tx714 hybrid yield trials under non-irrigation (College Station, 2015)								
Pedigree (σ_g^2)	33.8	29.7	49.0	45.4	18.4	14.0	32.0	9.6
Range[Test] (σ_C^2)	8.2	4.2	-	-	-	1.1	3.5	-
Row[Test] (σ_R^2)	-	-	5.4	5.1	3.7	17.5	34.9	33.5

Table 11. Continued

Trait	DTA (days)	DTS (days)	PHT (cm)	FLH (cm)	EHT (cm)	GM (%)	Tstwt (kg/hL)	GY (ton/ha)
Rep[Test] (σ_r^2)	3.4	17.5	0.7	-	5.4	0.9	-	4.6
Test (σ_T^2)	-	-	-	-	-	-	-	-
Residual (σ_ε^2)	54.6	48.6	45.0	49.5	72.6	66.4	29.6	52.3
H^2	0.6	0.6	0.7	0.7	0.3	0.3	0.7	0.3
$F_{3:4}$/ Tx714 hybrid yield trials under irrigation (College Station, 2015)								
Pedigree (σ_g^2)	4.1	6.6	38.2	42.0	34.1	44.8	31.5	2.7
Range[Test] (σ_C^2)	-	-	5.6	0.8	-	1.0	-	-
Row[Test] (σ_R^2)	12.1	8.8	2.3	-	-	20.5	13.9	-
Rep[Test] (σ_r^2)	1.1	2.6	5.8	10.6	1.1	-	-	-
Test (σ_T^2)	18.7	15.3	-	-	-	0.4	1.3	-
Residual (σ_ε^2)	64.1	66.8	48.2	46.6	64.8	33.4	53.3	97.3
H^2	0.1	0.2	0.6	0.6	0.5	0.7	0.5	0.1
$F_{4:5}$/Tx714 hybrid yield trials (Weslaco, 2015)								
Pedigree (σ_g^2)	0.6	0.0	28.9	24.4	23.1	na	na	na
Range (σ_C^2)	20.7	17.4	27.4	36.3	25.0	na	na	na
Row (σ_R^2)	-	0.2	1.1	-	-	na	na	na
Rep (σ_r^2)	39.3	39.4	-	-	-	na	na	na
Residual (σ_ε^2)	39.5	43.0	42.5	39.3	51.9	na	na	na
H^2	0.0	-	0.6	0.6	0.5	na	na	na

na:non-available data; "-" represents the factor was removed from the model.

Table 12. The percentage of total variance explained by each variance component and heritability on progeny-mean basis for each trait across all trials in population LH82/LAMA-YC.

Trait	DTA (days)	DTS (days)	PHT (cm)	FLH (cm)	EHT (cm)	GM (%)	Tstwt (kg/hL)	GY (ton/ha)
$F_{3:4}$ inbred trials (College Station, 2015)								
Pedigree (σ_g^2)	18.4	11.3	61.5	55.4	27.8	na	na	na
Range (σ_C^2)	2.9	0.1	0.3	1.2	1.4	na	na	na
Row (σ_R^2)	3.0	1.6	5.6	6.7	11.5	na	na	na
Rep (σ_r^2)	-	-	1.1	0.7	4.3	na	na	na
Residual (σ_ε^2)	75.7	87.0	31.5	36.1	55.0	na	na	na
H^2	0.3	0.2	0.8	0.8	0.5	na	na	na
$F_{3:4}$/ Tx714 hybrid yield trials under non-irrigation (College Station, 2015)								
Pedigree (σ_g^2)	17.5	11.2	53.5	50.0	9.4	11.7	80.2	54.6
Range[Test] (σ_C^2)	-	-	-	-	-	-	1.0	-
Row[Test] (σ_R^2)	15.1	9.8	15.1	6.3	14.5	12.9	10.2	19.3
Rep[Test] (σ_r^2)	16.2	1.5	7.9	16.7	12.3	4.7	-	-
Test (σ_T^2)	-	-	-	-	-	-	-	-
Residual (σ_ε^2)	51.3	77.5	23.6	27.0	63.8	70.7	8.6	26.2
H^2	0.4	0.2	0.8	0.8	0.2	0.3	1.0	0.8

Table 12. Continued

Trait	DTA (days)	DTS (days)	PHT (cm)	FLH (cm)	EHT (cm)	GM (%)	Tstwt (kg/hL)	GY (ton/ha)
$F_{3:4}$/ Tx714 hybrid yield trials under irrigation (College Station, 2015)								
Pedigree (σ_g^2)	20.5	14.9	45.8	41.0	4.1	28.6	30.2	22.6
Range[Test] (σ_C^2)	9.7	8.5	4.4	6.6	3.3	-	1.1	-
Row[Test] (σ_R^2)	23.7	-	3.7	3.5	-	42.9	19.3	3.1
Rep[Test] (σ_r^2)	-	3.6	-	-	2.1	1.5	-	-
Test (σ_T^2)	2.0	-	-	-	1.5	-	-	-
Residual (σ_ε^2)	44.1	73.0	46.1	48.8	89.0	27.0	49.4	74.3
h^2	0.5	0.3	0.7	0.6	0.1	0.7	0.6	0.4
$F_{4:5}$/Tx714 hybrid yield trials (Weslaco, 2015)								
Pedigree (σ_g^2)	1.3	15.1	30.5	30.3	28.0	na	na	na
Range (σ_C^2)	1.3	1.0	-	-	-	na	na	na
Row (σ_R^2)	21.0	12.8	8.5	7.0	4.8	na	na	na
Rep (σ_r^2)	-	-	-	-	-	na	na	na
Residual (σ_ε^2)	76.5	71.1	61.0	62.7	67.3	na	na	na
H^2	0.0	0.3	0.5	0.5	0.5	na	na	na

na: non-available data; "-" represents the factor was removed from the model.

Correlation of traits

For $F_{3:4}$ inbred trials (College Station, 2015), there was a strong positive correlation between days to anthesis and days to silking in each population; plant height was also strongly positively correlated with flag leaf height and ear height (Tables 13, 14 & 15). Flowering time and plant height were negatively correlated in two tropically derived populations (Ki3/NC356 and Tx740/NC356), but positively correlated in the temperate × tropical derived population (LH82/LAMA-YC).

Correlation estimates for all traits collected from $F_{3:4}$/Tx714 hybrid yield trials were summarized in Tables 16, 17&18. In the Ki3/NC356 population, grain yield was positively correlated with plant height, flag leaf height and ear height and it was negatively correlated with flowering time and grain moisture at irrigated and non-irrigated conditions (Table 16). In a previous study, Barrero Farfan et al. (2013) also found that plant height, ear height, plant population and test weight were positively correlated with grain yield in commercial temperate hybrids with stronger effects observed in the rest of Texas (which includes College Station) than in the Texas High Plains, and these were still stronger than typically observed in the Midwestern US. Therefore, a hypothesis had been proposed that taller plants in Ki3/NC356 population would be correlated with higher grain yield in some Texas environments and this was confirmed in this study.

Table 13. Correlation estimates for multiple traits collected for Ki3/NC356 $F_{3:4}$ inbred trials in College Station, TX, 2015. The lower half diagonal correspond to the genetic correlations; the upper diagonal correspond to the phenotypic correlations.

	DTA (days)	DTS (days)	PHT (cm)	FLH (cm)	EHT (cm)
DTA (days)		0.88***	-0.28***	-0.27***	-0.11*
DTS (days)	0.87***		-0.31***	-0.31***	-0.14**
PHT (cm)	-0.06	0.00		0.95***	0.65***
FLH (cm)	-0.05	-0.02	0.93***		0.69***
EHT (cm)	0.15***	0.19	0.55***	0.60***	

*P<0.05;** P<0.01; ***P<0.001.

Table 14. Correlation estimates for multiple traits collected for Tx740/NC356 $F_{3:4}$ inbred trials in College Station, TX, 2015. The lower half diagonal correspond to the genetic correlations; the upper diagonal correspond to the phenotypic correlations.

	DTA (days)	DTS (days)	PHT (cm)	FLH (cm)	EHT (cm)
DTA (days)		0.60***	-0.45***	-0.41***	-0.09
DTS (days)	0.59***		-0.57***	-0.54***	-0.35***
PHT (cm)	-0.32***	-0.43***		0.95***	0.57***
FLH (cm)	-0.27***	-0.37***	0.93***		0.61***
EHT (cm)	0.15	-0.11	0.51***	0.58***	

*P<0.05;** P<0.01; ***P<0.001.

Table 15. Correlation estimates for multiple traits collected for LH82/LAMA-YC $F_{3:4}$ inbred trials in College Station, TX, 2015. The lower half diagonal correspond to the genetic correlations; the upper diagonal correspond to the phenotypic correlations.

	DTA (days)	DTS (days)	PHT (cm)	FLH (cm)	EHT (cm)
DTA (days)		0.53***	0.20***	0.17**	0.14*
DTS (days)	0.40***		0.13	0.12	0.13
PHT (cm)	0.30***	0.08		0.91***	0.60***
FLH (cm)	0.27***	0.07	0.93***		0.63***
EHT (cm)	0.28***	0.05	0.65***	0.67***	

*P<0.05;** P<0.01; ***P<0.001.

Table 16. Correlation estimates for multiple traits collected for Ki3/NC356 $F_{3:4}$/Tx714 hybrid yield trials in College Station, TX, 2015. The lower half diagonal correspond to the trials under irrigated condition; the upper diagonal correspond to the trails under non-irrigated condition.

	DTA (days)	DTS (days)	PHT (cm)	FLH (cm)	EHT (cm)	GM (%)	Tstwt (kg/hL)	GY (ton/ha)
DTA (days)		0.95***	-0.32***	-0.27***	-0.31***	0.16*	-0.18*	-0.50***
DTS (days)	0.90***		-0.42***	-0.37***	-0.41***	0.21**	-0.18*	-0.59***
PHT (cm)	-0.14**	-0.16***		0.96***	0.86***	-0.17*	0.24**	0.7***
FLH (cm)	-0.09	-0.10*	0.88***		0.85***	-0.14*	0.24**	0.66***
EHT (cm)	0.02	-0.01	0.66***	0.69***		-0.20**	0.22*	0.64***
GM (%)	0.10	0.08	-0.16**	-0.13*	-0.11*		-0.14	-0.32***
Tstwt (kg/hL)	0.12*	0.11*	-0.06	-0.12*	-0.12*	-0.05		0.34***
GY (ton/ha)	-0.26***	-0.32***	0.43***	0.36***	0.24***	-0.17**	-0.16**	

*P<0.05;** P<0.01; ***P<0.001.

Table 17. Correlation estimates for multiple traits collected for Tx740/NC356 $F_{3:4}$/Tx714 hybrid yield trials in College Station, TX, 2015. The lower half diagonal correspond to the trials under irrigated condition; the upper diagonal correspond to the trails under non-irrigated condition.

	DTA (days)	DTS (days)	PHT (cm)	FLH (cm)	EHT (cm)	GM (%)	Tstwt (kg/hL)	GY (ton/ha)
DTA (days)		0.81***	0.20*	0.25**	0.16	-0.05	0.18	0.00
DTS (days)	0.81***		0.13	0.20*	0.19*	0.03	0.21	-0.04
PHT (cm)	-0.04	-0.01		0.89***	0.44***	0.12	-0.28**	0.21*
FLH (cm)	-0.02	-0.04	0.89***		0.49***	0.06	-0.16	0.14
EHT (cm)	0.00	0.00	0.47***	0.51***		0.19	0.16	0.16
GM (%)	-0.08	-0.15	-0.12	-0.07	-0.14		0.04	0.00
Tstwt (kg/hL)	0.03	0.04	-0.15	-0.23**	-0.12	-0.20*		0.09
GY (ton/ha)	0.09	0.04	0.01	0.07	0.12	0.19*	-0.24**	

*P<0.05;** P<0.01; ***P<0.001.

Table 18. Correlation estimates for multiple traits collected for LH82/LAMA-YC $F_{3:4}$/Tx714 hybrid yield trials in College Station, TX, 2015. The lower half diagonal correspond to the trials under irrigated condition; the upper diagonal correspond to the trails under non-irrigated condition.

	DTA (days)	DTS (days)	PHT (cm)	FLH (cm)	EHT (cm)	GM (%)	Tstwt (kg/hL)	GY (ton/ha)
DTA (days)		0.89***	0.17	0.14	-0.04	-0.18	-0.46***	-0.22
DTS (days)	0.72***		0.28*	0.25*	0.02	-0.17	-0.54***	-0.21
PHT (cm)	0.04	0.04		0.93***	0.63***	0.04	-0.48***	0.22
FLH (cm)	0.05	0.08	0.90***		0.65***	0.06	-0.41**	0.26*
EHT (cm)	0.09	0.05	0.57***	0.63***		0.08	-0.25	0.22
GM (%)	0.04	0.01	0.17*	0.19*	0.15		0.25	0.19
Tstwt (kg/hL)	0.16*	0.10	-0.04	-0.05	0.04	-0.1		-0.07
GY (ton/ha)	-0.15	-0.19*	0.22**	0.21**	0.16*	0.04	-0.06	

*P<0.05;** P<0.01; ***P<0.001.

Validating QTVs' effects by ANOVA analysis

All traits mean values and genotype data of the three target QTVs were fitted into the multiple regression model (Eq. 3 described in Methods and Materials). ANOVA analysis of each QTV for each trait across all tests are presented in Tables 19, 20 and 21.

It was previously estimated that QTV1, QTV2 and QTV3 explained 3- 5% variation of grain yield at irrigated and non-irrigated testcross hybrid trials, and the allelic effects of these QTVs ranged from 0.14 to 0.59 ton/ha; QTV2 was also identified for plant height, explaining 4.6 to 5% of phenotypic variation with the effect ranged from 5.3 to 5.6 cm; and for days to anthesis and days to silk with the effect ranging from 1.3 to 1.8 days (Farfan et al. 2015). The data from this study often supported the findings that these SNPs were significant, but these had different absolute effect sizes than what was estimated in Farfan et al.'s (2015) GWAS study and also occasionally affected different traits.

The Ki3/NC356 population was the only population with all three QTV segregating between the two parental lines (Table 4). In $F_{3:4}$ inbred trials, QTV1, QTV2 and QTV3 were found to be significant for days to anthesis; the additive effects for these three QTVs ranged from 0.2-0.5 (day) and the dominance effects were from 0.4-0.5 (day). For days to silk, only QTV1 and QTV3 were found to be significant in $F_{3:4}$ inbred trials and the additive effects were 0.4 and 0.7 (day), respectively. In addition, QTV1 and QTV3 were found to be significant for both plant height and flag leaf height; the alleles with positive effects at these two loci increased plant height from 1.4 cm to 4.0

cm and flag leaf height from 1.8 cm to 3.0 cm across all tests over two years. The alleles that increased height came from parent line NC356, which were in agreement with the previous finding about the alleles of positive effects (Table 1) (Farfan et al. 2015). In $F_{3:4}$/Tx714 hybrid yield trials under the non-irrigated condition, QTV1 was found to be significant for plant height and grain test weight; the allele from NC356 increased plant height 1.9 cm and grain test weight 0.2 kg/hL in the testcross hybrids. In $F_{3:4}$/Tx714 hybrid yield trials under irrigation, QTV3 was identified as significant for plant height and grain test weight; the allele from NC356 increased plant height 1.4 cm and test weight 0.14 kg/hL. That the NC356 alleles had positive effects at QTV1 and QTV3 loci was in agreement with the previous GWAS results (Table 1) (Farfan et al. 2015). An important and interesting finding was that plant height was highly positive correlated with grain test weight and grain yield in $F_{3:4}$/Tx714 hybrid yield trials (Table 16); this suggested taller plants possessing the alleles at QTV1 and QTV3 from NC356 would have the potential to improve grain yield in Ki3/NC356 population.

In the other two populations, due to the smaller population size and substantial field variation due to flooding in these tests, there were few consistent and significant QTVs' effects validated (Table 20 and 21).

Table 19. Summary of QTVs' multiple regression analysis for all traits in Ki3/NC356 population (College Station, Weslaco, 2014~ 2015). The lower slash is additive effect value of the significant QTV; the upper slash is dominance effect value pf the significant QTV.

	DTA (days)	DTS (days)	PHT (cm)	FLH (cm)	EHT (cm)	GM (%)	Tstwt (kg/hL)	GY (ton/ha)
	$F_{3:4}$ inbred nursery (Weslaco, 2015)							
QTV1	na	na	3.6/1.1*	3.1/0.9*	-	na	na	na
QTV2	na	na	ns	ns	-	na	na	na
QTV3	na	na	-3.0/4.7*	-3.1/3.4*	-	na	na	na
	$F_{3:4}$ inbred trials (College Station, 2015)							
QTV1	0.5/0.0***	0.7/0.2***	4.0/2.0***	2.5/1.8***	1.0/3.1***	na	na	na
QTV2	0.2/-0.4*	ns	-0.1/3.3*	-0.2/3.2*	ns	na	na	na
QTV3	0.4/-0.5***	0.4/-0.2*	-2.2/3.8***	-1.8/3.9***	-	na	na	na
	$F_{3:4}$/Tx714 hybrid yield trials under non-irrigation (College Station, 2015)							
QTV1	0.4/-0.4*	ns	1.9/0.3*	ns	ns	ns	0.20/0.28*	ns
QTV2	-	-	-	ns	ns	-0.001/-0.02*	ns	-
QTV3	ns	-	ns	ns	-	ns	ns	ns
	$F_{3:4}$/Tx714 hybrid yield trials under irrigation (College Station, 2015)							
QTV1	ns	ns	2.3/0.1***	1.8/-0.4**	ns	0.22/-0.13**	ns	ns
QTV2	ns	-0.3/-0.2**	ns	-	-	-0.22/0.39***	ns	-
QTV3	-	ns	-1.4/0.4*	ns	ns	-	-0.14/-0.62***	ns
	$F_{4:5}$/Tx714 hybrid yield trials (Weslaco, 2015)							
QTV1	ns	ns	ns	-	ns	na	na	na
QTV2	ns	ns	ns	ns	ns	na	na	na
QTV3	ns	ns	-	-	-	na	na	na

*P<0.05;** P<0.01; ***P<0.001; ns: non-significant; na: non-available; "-": being excluded from the mixed model.

Table 20. Summary of QTVs' multiple regression analysis for all traits in Tx740/NC356 tests (College Station, Weslaco, 2014~ 2015). The lower slash is additive effect value of the significant QTV; the upper slash is dominance effect value pf the significant QTV.

	DTA (days)	DTS (days)	PHT (cm)	FLH (cm)	EHT (cm)	GM (%)	Tstwt (kg/hL)	GY (ton/ha)
	$F_{3:4}$ inbred nursery (Weslaco, 2015)							
QTV1	na	na	ns	ns	ns	na	na	na
QTV2	na	na	ns	ns	ns	na	na	na
	$F_{3:4}$ inbred trials (College Station, 2015)							
QTV1	-0.1/0.8*	0.7/1.3**	ns	ns	-	na	na	na
QTV2	-	-	-	-	ns	na	na	na
	$F_{3:4}$/Tx714 hybrid yield trials under non-irrigation (College Station, 2015)							
QTV1	0.2/0.6*	-	3.0/3.2**	2.0/4.3**	-	0.05/0.06**	-	-
QTV2	-	-	-	-	-	ns	ns	0.4/-0.3**
	$F_{3:4}$/Tx714 hybrid yield trials under irrigation (College Station, 2015)							
QTV1	-	-	ns	ns	ns	-	ns	-
QTV2	ns	-	-	ns	-	-	-0.19/0.51*	-
	$F_{4:5}$/Tx714 hybrid yield trials (Weslaco, 2015)							
QTV1	-	-	-	ns	ns	na	na	na
QTV2	-	-	4.0/4.5*	3.9/0.7*	-	na	na	na

*P<0.05;** P<0.01; ***P<0.001; ns: non-significant; na: non-available; "-": being excluded from the mixed model.

Table 21. Summary of QTVs' multiple regression analysis for all traits in LH82/LAMA-YC tests (College Station, Weslaco, 2014~ 2015). The lower slash is additive effect value of the significant QTV; the upper slash is dominance effect value pf the significant QTV.

	DTA (days)	DTS (days)	PHT (cm)	FLH (cm)	EHT (cm)	GM (%)	Tstwt (kg/hL)	GY (ton/ha)
	$F_{3:4}$ inbred nursery (Weslaco, 2015)							
QTV1	na	na	ns	ns	ns	na	na	na
QTV2	na	na	ns	ns	ns	na	na	na
	$F_{3:4}$ inbred trials (College Station, 2015)							
QTV1	-	ns	-0.7/6.1***	-0.3/4.5**	ns	na	na	na
QTV2	-	ns	-	-	ns	na	na	na
	$F_{3:4}$/Tx714 hybrid yield trials under non-irrigation (College Station, 2015)							
QTV1	-	-	-	-	-	-	0.66/-0.02*	-
QTV2	-	-	-	-	-	0.11/0.03*	-	ns
	$F_{3:4}$/Tx714 hybrid yield trials under irrigation (College Station, 2015)							
QTV1	-	-	-	-	-	ns	ns	ns
QTV2	-	0.2/0.2*	ns	ns	-	-	-	ns
	$F_{4:5}$/Tx714 hybrid yield trials (Weslaco, 2015)							
QTV1	-	-	2.4/3.0*	ns	3.2/-3.0***	na	na	na
QTV2	-	-	-1.4/4.4*	ns	ns	na	na	na

*P<0.05;** P<0.01; ***P<0.001; ns: non-significant; na: non-available; "-": being excluded from the mixed model.

Conclusion

In this study, three bi-parental linkage populations were used to validate the effects of three QTVs, which were identified in previous GWAS (Farfan et al. 2015). By single marker analysis, QTV1 and QTV3 were consistently confirmed as significant for plant height across $F_{3:4}$ inbred and the corresponding testcross hybrid trials in the biggest population Ki3/NC356; and the alleles with positive effects at these two loci were from NC356. In addition, grain yield was positively correlated with plant height in $F_{3:4}$/Tx714 hybrid yield trials, which were in agreement with previous study of Texas AgriLife Corn Performance Trials (Barrero Farfan et al. 2013) and suggested that selecting higher plants in population Ki3/NC356 could improve grain yield.

CHAPTER III

HIGH-DENSITY LINKAGE MAP CONSTRUCTION AND MAPPING OF AGRONOMIC TRAITS IN TROPICAL MAIZE (ZEA MAYS L.)

Introduction

Maize (*Zea mays* L.) is one of the most important and widely grown crops in the world and it has an essential role in plant biology and quantitative genetics. Due to an outcrossing nature, maize has more genetic diversity than the self-pollinated crops, like wheat and rice, and likely more phenotypic diversity. Maize has extensive germplasm resources throughout the world (i.e. CIMMTY, USDA-ARS). Arisen from teosinte within the past 10,000 years, maize has undergone several rounds of detectible genome duplication events, resulting in rich genetic variation (Blanc and Wolfe 2004; Paterson, Bowers, and Chapman 2004; Buckler and Stevens 2005). The maize genome is about 2.4 gigabases, nearly 85% of which is made up of transposable elements (TEs) (Schnable et al. 2009) and one of the most recent genome assembly's predicts over 36,413 genes were predicted based on B73 RefGen_v3 (http://ensembl.gramene.org/Zea_mays/Info/Annotation/).

Most economically important maize traits are complex, such as flowering time, plant height, yield, etc. and are controlled by a large number of small-effect genes. To characterize the genetic architecture of complex quantitative traits, many quantitative trait loci (QTL) analyses have been performed in maize. However, the QTL in different

maize populations are dependent upon the genetic variation (G) segregating in the population, the environments (E) that the population is evaluated in, the interactions between these factors (G × E), and the statistical and technical limitations to detection. Therefore, no one QTL mapping study can ever be definitive, instead each is designed to address a specific set of questions but taken together they help to build the body of knowledge for the genetic architecture of quantitative traits in maize. The statistical and technical limitations to detecting QTL largely depends on the population type and size. Most QTL mapping studies are performed in advanced immortalized (permanent) populations, such as recombinant inbred lines (RILs) and more recently, the maize NAM population (H-Y Hung et al. 2012; McMullen et al. 2009) and MAGIC populations (Dell'Acqua, D. M. D. Gatti, et al. 2015; Holland 2015) have been created for high definition QTL mapping, which allows the detection of more minor effect QTLs (Buckler et al. 2009; Cook et al. 2012; Peiffer et al. 2014). A large set of early generation populations, such as F_2, has also been used and is able to detect QTLs efficiently (Chen et al. 2014).

With the availability of maize genome sequence information (Schnable et al. 2009), tens of millions of single nucleotide polymorphisms (SNPs) have been discovered from various maize lines (Chia et al. 2012) and applied as high- throughput genotyping arrays (Ganal et al. 2011; Yan et al. 2010). Compared with the historical molecular markers, like RFLP, AFLP and SSR, SNP markers are more plentiful for high-density genetic mapping and useful to increase the mapping resolution and efficiency. More recently, a few high-density linkage mapping studies were reported in maize and when

combined with larger population size, greatly improve the ability of QTLs detection (Buckler et al. 2009; Guo et al. 2014; Pan et al. 2012; Peiffer et al. 2014; Raihan et al. 2016).

To date, over 2,200 QTLs have been reported in maize (http://maizegdb.org/) in the subset of populations uploaded to MaizeGDB alone. However, the vast majority of reported QTLs for maize traits were identified in unique experimental populations from temperate maize germplasm. A more limited number of QTL mapping studies have been performed in tropical maize germplasm (Bohn et al. 1996; Groh et al. 1998; Khairallah et al. 1998; Lima et al. 2006; Mangolin et al. 2004; Messmer et al. 2009; Ribaut et al. 1996, 1997; Sibov et al. 2003; Trachsel et al. 2009) and most previous QTL studies were conducted with fewer molecular markers and individuals than would currently be advised, increasing the statistical and technical limitations. To our knowledge, no QTL linkage mapping study has yet been reported in tropical derived maize germplasms using high density genotyping, which is one of multiple novelties of this study.

Texas is a primary maize producer in the Southern United States and many of the fields in south and central Texas regions are sub-tropical like in climate and grown as dryland locations, which are very different from the conditions of the U.S. Corn Belt (Barrero Farfan et al. 2013). In contrast to the steadily increasing grain yield over years in Corn Belt, Texas farmers face some issues that seriously impede maize production, such as drought stress, aflatoxin contamination and high night temperatures. A major reason for these problems is the use of unadapted temperate derived germplasm for

subtropical environments in Southern Texas. Currently most widely sold hybrids are well adapted to temperate environments in the Midwestern United States, and contain 5% tropical germplasm (Goodman, M.M. 1999). Tropical maize germplasm is notable for its broader genetic diversity (Lanza et al. 1997; Reif et al. 2004; Wen et al. 2012), which is a more promising source to improve maize production in the Southern United States and throughout the sub-tropics. Therefore, QTL mapping in tropical derived maize populations could identify novel QTLs and broaden the potential for maize production in the Southern U.S..

A limitation of many past QTL mapping studies has been the evaluation of inbred line progeny per se. From a maize breeding perspective, it makes more sense to test for QTLs in the corresponding testcross hybrids although few linkage mapping studies (Mayfield et al. 2011) and association mapping studies (Farfan et al. 2015; Warburton et al. 2015) have done so. In the present study, three different bi-parental linkage populations were selected to satisfy three primary criteria: 1) they needed to be Texas adapted and breeding relevant; 2) similarly they needed to be crosses within and not between heterotic groups; 3) they needed to be segregating for at least two of three QTVs detected in Farfan et al. 2015 (Chen et al. Chapter II); and finally 4), they needed to be already at the F_2 stage to fit into a graduate student research timeline. Therefore, in this study, QTL mapping was conducted in $F_{3:4}$ inbred lines per se progeny and their corresponding $F_{3:4}$/Tx714 testcross hybrids in each linkage population.

Considering the novelties of this present study (i.e. tropical maize germplasm, Southern US environment, high-density SNP assay, and three linkage populations sharing partial common genetic background), there were several applied objectives we sought to address in this study: (1) to detect novel QTLs for flowering time, plant height, grain yield and other relevant agronomic traits in Texas tropical maize populations; (2) to identify some consistent QTLs across $F_{3:4}$ progenies and their corresponding $F_{3:4}$/Tx714 hybrids in each linkage population; (3) to identify some consistent QTLs across three linkage population. We also discovered several technical goals that would be relevant to other studies including (1) to evaluate various software useful for building a genetic map and detecting QTL from a high density SNPs array in an $F_{3:4}$ population; (2) to evaluate the consistency of using best linear unbiased estimators (BLUEs) versus best linear unbiased predictors (BLUPs) for detecting QTLs in each population.

Methods and Materials

Experimental populations

In the previous GWAS (Farfan et al. 2015), three quantitative trait variants (QTVs) were identified as significant, each explaining 3-5% phenotypic variation of grain yield. Initially, to validate these QTVs effects on maize grain yield and other agronomic traits, three linkage populations were advanced in part because they segregated at the three QTVs for validation and also they were promising breeding crosses in our program (Table 4 in Chapter II). The three linkage populations included (1) Ki3/NC356, (2) Tx740/NC356 and (3) LH82// (LAMA2002-12-1-B-B-B-

B/LAMA2002-1-5-B-B-B-B)-3-2-B-1-B3-B. (LAMA2002-12-1-B-B-B-B/LAMA2002-1-5-B-B-B-B) is a breeding line related to Tx740 (Mayfield et al. 2012), and will be referred to as LAMA-YC in this study. The first two populations (1) and (2) used parents that were fully tropically derived while the population (3) was derived from the temperate (expired plant variety protection line) × tropical cross.

Field experiment design and phenotypic evaluation

The field experiment design and phenotypic measurements were the same as described in Chapter II. Through selfing the $F_{2:3}$ progeny, $F_{3:4}$ individuals were produced in College Station, TX (CS14) in 2014 summer. Subsequently, $F_{3:4}$ progeny of each population were grown and evaluated at the winter nursery in Weslaco, TX, 2014 (WE14); each $F_{3:4}$ line was crossed to a Texas adapted inbred tester Tx714 (Betrán et al. 2004), the same tester used in previous GWAS (Farfan et al. 2015) to produce $F_{3:4}$ testcross hybrids ($F_{3:4}$/Tx714). In the summer of 2015, $F_{3:4}$/Tx714 hybrid yield trials were evaluated for phenotype in both early (irrigated) and late (non-irrigated) planted trials in College Station, TX (CS15 yield trials) and as $F_{3:4}$ inbred trials.

The $F_{3:4}$ inbred progenies and $F_{3:4}$/Tx714 hybrid yield trials were located on Texas A&M University Farm in Burleson, TX on a ships clay loam soil. Each experimental plot was 7.62 meters long and 76.2 centimeters wide. Depending on the available seed amount, the $F_{3:4}$/Tx714 testcross hybrids were laid out in the field for four replications, two replications or one replication (Table A1); the commercial line DK64-69 was used as a check. Each of $F_{3:4}$ inbred progenies was grown in two replications in

inbred yield trials under irrigation. For the nursery of population development, each plot was 3.04 meters long and 76.2 centimeters wide; each progeny was grown in one single plot.

Days to silk (DTS, female flowering) and days to anthesis (DTA, male flowering) were estimated by 50% of the plants within each plot showing silks or shedding pollen. After pollination, one average-performing plant within each individual plot was selected for the measurements of plant height (PHT, soil to tip of tassel), flag leaf height (FLH, soil to flag leaf node) and ear height (EHT, soil to top ear node) in centimeter. A HM-1000B Grain Gauge mounted on the John Deere (Moline, IL) 3300 combine measured grain moisture (GM), expressed as the percentage of the test weight; test weight (Tstwt) was determined as kg/hl. The grain yield value (GY) was calculated from the plot weight and was standardized to 15.5% moisture and expressed as ton/hectare.

Genotyping

Genomic DNAs were extracted within each $F_{3:4}$ progeny from eight bulked seedlings (to capture segregation distortion and with the genotypes equivalent to the single progenitor F_3 plant) and the parental lines using CTAB method (Chen and Ronald 1999). With technical and in-kind support from AgReliant Genetics LLC, all $F_{3:4}$ progeny of three linkage populations were genotyped by Infinium®assays using 17,344 single-nucleotide polymorphisms (SNPs); the SNPs showing polymorphism between two parental lines were initially used for linkage map construction and QTL mapping.

Statistical analysis

Basic statistical analysis for each trait in each test was conducted as detailed in Chapter II. All the phenotypic data was analyzed using JMP® Pro 12.0.1(SAS Institute Inc., Cary, NC, 1989-2007.). A residual maximal likelihood (REML) approach was applied to conduct statistical analysis. A random linear model (Eq. 1) was used to fit all the data within each test to estimate the variance components. Best linear unbiased predictions (BLUPs) of each trait for each $F_{3:4}$ inbred line and $F_{3:4}$/Tx714 hybrid were predicted from a random effects model (Eq. 1); Best linear unbiased estimates (BLUEs) of each trait for individual $F_{3:4}$ line and their testcross hybrids were also estimated from a mixed model by considering each genotype as a fixed factor in Eq. 1. Both BLUPs and BLUEs for all traits of individual $F_{3:4}$ progeny and their testcross hybrids were used to perform QTL mapping. Broad sense heritability on progeny-mean basis was calculated to determine how much of the phenotypic variation was attributed to genetic variance using (Eq. 2). Traits correlation estimates were plotted by using 'corrplot' package in R (Wei and Simko 2016).

Linkage map construction and QTL mapping

Available and popular software packages are currently not well suited for making high-density genetic maps in the F_3 generation, especially across populations. To minimize the limitations in individual software, in this study we used multiple programs to create genetic maps and perform QTL analysis.

Due to high-density SNP markers and multiple populations, R/qtl (Broman et al. 2003) was applied for both linkage map development and QTL mapping and its data cleaning functions were very helpful. Within each population, the individuals with >10% missing genotype data were omitted and the SNP markers with >10% missing value were dropped by the function "subset.cross ()" and "drop.markers ()" respectively. The function "geno.table ()' was used to inspect the segregation patterns in each cross, which calculated the genotype frequencies and also a p-value to test the departure from the expected segregation ratio; because the genotype frequencies of F_3 generation (2:1:2) was not available in the R/qtl package, all these three populations were treated as F_2 intercross type, which the ratio of genotype frequencies is 1:2:1. At the $p < 0.05$ level, the highly distorted segregation markers were considered as bad genotyping data and excluded from further analysis. The function "formLinkageGroups ()" was used to group the markers into linkage groups based on an estimated recombination frequency ≤ 0.30 and a LOD score ≥ 3.5. The function "orderMarkers ()" with the arguments "use.ripple=TRUE" and "map.function='kosambi' " was used to establish the initial appropriate order within each linkage group. The function "countXO ()" was used to investigate the number of crossovers in each individual; the particular individuals with high crossover number were deleted from the populations by function "subset (, ind = (countXO () < average crossover number))". At last, the function "orderMarkers ()" with the arguments "use.ripple=TRUE" and "map.function='kosambi' " was used again to establish the optimal order within each linkage group (R script was provided in Appendix).

We encountered issues with map expansion and large disordered blocks, confirmed by comparing the maps of the three populations, which could not be resolved in R/qtl. Therefore the dataset cleaned using R/qtl, in which highly distorted segregation SNP markers and individual progenies with high crossover numbers were excluded and exported as clean dataset for further analysis (Table 22).

IciMapping version 4.1.0.0 (http://www.isbreeding.net/) was then used to finish the construction of the linkage map and to perform QTL mapping. Three tools were involved: (1) BIN, which was used to remove redundant markers (i.e. markers are perfectly linked in a genomic region); (2) MAP, constructing genetic map through grouping by LOD=3.00, ordering by the "nnTwoOpt" algorithm and rippling by "SARF" criterion with window size 5; (3) BIP, deleting the missing phenotype and mapping QTLs in bi-parental populations by the "ICIM-ADD" method. Finally, the threshold for detecting the existence of a significant QTLs for each trait in each population was obtained by 1000 times permutation at a significant level of P =0.05.

Table 22. Summary of the clean data used for linkage map construction.

Pop.	Original population size	Total number of polymorphic SNPs	Number of individuals in linkage map	Number of SNPs in linkage map generated by R/qtl
1	239	5,913	174	3,383
2	110	5,795	87	5,073
3	178	6,439	150	4,297

Results and Discussion

Phenotypic data analysis

Rain in May and June, 2015, caused the Brazos River to overflow and all field trials were submerged for multiple days at Texas A&M University Farm in Burleson, TX. In $F_{3:4}$ inbred trials, the population LH82/LAMA-YC were more greatly affected by flood damage than the other two populations due to field locations. Among the $F_{3:4}$/Tx714 hybrids, the irrigated field was not irrigated ever and the primary difference between non-irrigated tests and irrigated tests for each population was the planting date; the irrigated tests were planted on March 2nd, 2015, and the non-irrigated tests were planted on March 16th, 2015. Flood

Phenotypic data from $F_{3:4}$ inbred trials and from $F_{3:4}$/Tx714 hybrid trials of each population were analyzed and the basic statistical data is summarized in Table 23 & 24. It was noticeable that broad sense heritability on progeny-mean basis of flowering time (DTA and DTS) in population LH82/LAMA-YC $F_{3:4}$ inbred trials was much lower than the other two populations (Table 23). Although flood damage resulted in more random error for these traits in the field, there was less phenotypic variation. This was surprising because LH82 was an ex-PVE line from temperate germplasm while LAMA-YC was derived from tropical germplasm; there were many morphological polymorphisms between these two parental lines but few phenotypic differences among their $F_{3:4}$ progenies. This suggested there were many unlinked loci with infinitesimal very small

effects (i.e. an infinitesimal model) controlling flowering time in the population derived from the cross between LH82/LAMA-YC.

Best linear unbiased estimates (BLUEs) and best linear unbiased predictions (BLUPs) for each individual trait in each test were summarized in supplementary materials (Table A2.)

Table 23. Summary statistics of parental lines and their $F_{3:4}$ inbred progenies for each trait by each population (College Stations, 2015).

Trait	Parent means		Min.	Max.	Mean ± S. D.	H^2
	Ki3	NC356	$F_{3:4}$ inbred			
DTA (days)	88.5	87.5	77.0	92.0	85.4 ± 2.5	0.86
DTS (days)	89.5	90.0	79.0	94.0	86.1 ± 2.7	0.78
PHT (cm)	124.5	147.3	109.2	190.5	149.5 ± 16.9	0.79
FLH (cm)	96.5	113.0	78.7	157.5	117.3 ± 15.6	0.75
EHT (cm)	33.0	52.1	20.3	81.3	51.4 ± 11.3	0.56
	Tx740	NC356	$F_{3:4}$ inbred			
DTA(days)	92	88.5	84.0	92.0	88.4 ±1.8	0.81
DTS(days)	98.5	96.5	83.0	103.0	94.3±4.2	0.66
PHT(cm)	123.2	113	101.6	167.6	131.2 ±14.6	0.68
FLH(cm)	95.3	82.6	73.7	132.1	98.9 ±13.3	0.68
EHT(cm)	39.4	35.6	22.9	66.0	44.8 ±9.2	0.74
	LH82	LAMA-YC	$F_{3:4}$ inbred			
DTA(days)	78.0	93.0	84.0	87.0	85.3 ± 0.7	0.33
DTS(days)	80.5	98.0	85.0	88.0	86.8 ± 0.6	0.21
PHT(cm)	88.9	119.4	76.2	154.9	114.0 ± 14.0	0.80
FLH(cm)	64.8	95.3	53.3	116.8	85.3 ± 11.4	0.75
EHT(cm)	21.6	29.2	12.7	50.8	30.5 ± 7.4	0.50

Mean: arithmetic mean value; Min.: minimum value; Max.: maximum value; H^2: broad sense heritability on progeny-mean basis.

Table 24. Summary statistics of $F_{3:4}$/Tx714 hybrid yield trials of each trait by each population (College Station, 2015).

Trait	Late planted yield trials				Early planted yield trials			
	Min.	Max.	Mean ± S. D.	H^2	Min.	Max.	Mean ± S. D.	H^2
Ki3/NC356								
DTA (days)	65	79	71.1 ± 2.9	0.73	74	82	78.3 ± 1.6	0.25
DTS (days)	67	82	73.5 ± 3.1	0.69	76	86	80.7 ± 1.8	0.35
PHT (cm)	193	269.2	236.5 ± 17.9	0.80	198.1	246.4	223.5 ± 10.5	0.46
FLH (cm)	139.7	231.1	189.9 ± 18.9	0.73	154.9	213.4	182.8 ± 10.8	0.48
EHT (cm)	58.4	134.6	99.0 ± 16.4	0.61	63.5	124.5	94.1 ± 10.8	0.46
GM (%)	11.75	12.45	12.12 ± 0.13	0.59	10.26	16.46	13.21 ± 1.20	0.71
Tstwt (kg/hL)	54.32	61.32	57.80 ± 1.30	0.87	55.23	62.9	59.07 ± 1.42	0.62
GY (ton/ha)	3.5	11.1	7.5 ±1.6	0.25	3.1	14.1	8.9 ± 2.1	0.41
	Tx740/NC356							
DTA (days)	65	71	68.1 ± 1.1	0.55	78	81	79.23 ±0.85	0.11
DTS (days)	68	76	71.7 ± 1.3	0.55	79	86	81.98 ±1.32	0.16
PHT (cm)	236.2	279.4	258.2 ± 9.3	0.69	213.4	256.5	234.81 ±8.77	0.61
FLH (cm)	190.5	231.1	209.7 ± 9.0	0.65	170.2	210.8	190.67 ±8.27	0.64
EHT (cm)	86.4	139.7	110.6 ± 9.6	0.34	76.2	124.5	99.57 ±9.81	0.51
GM (%)	11.73	12.7	12.19 ± 0.19	0.30	10.37	15.37	12.92 ±1.08	0.73
Tstwt (kg/hL)	54.46	60.83	57.56 ± 1.29	0.68	55.04	61.65	58.74 ±1.37	0.54
GY (ton/ha)	4.8	10.2	7.6 ± 1.2	0.27	7.63	13.91	10.79 ±1.24	0.05
	LH82/LAMA-YC							
DTA (days)	65	69	66.7 ± 1.3	0.41	74	77	75.7 ± 0.9	0.48
DTS (days)	67	72	69.5 ± 1.4	0.22	77	80	78.4 ± 0.8	0.29
PHT (cm)	215.9	264.2	235.7 ± 12.1	0.82	185.4	241.3	214.1 ± 10.8	0.67
FLH (cm)	170.2	218.4	192.11 ± 11.94	0.79	149.9	198.1	174.3 ± 9.7	0.63
EHT (cm)	76.2	127	100.3 ± 10.8	0.23	68.6	106.7	88.8 ± 8.4	0.08
GM (%)	11.62	13.03	12.32 ± 0.27	0.25	9.47	14.56	11.82 ± 1.15	0.67
Tstwt (kg/hL)	54.38	59.93	57.02 ± 1.36	0.95	54.32	61.8	58.03 ± 1.41	0.55
GY (ton/ha)	4.6	10.7	8.0 ± 1.5	0.81	5.4	13	9.3 ± 1.7	0.38

Mean: arithmetic mean value; Min.: minimum value; Max.: maximum value; H^2: broad sense heritability on progeny-mean basis.

Correlation of traits

For the traits correlated in $F_{3:4}$ progenies, a strong positive correlation between DTA and DTS was identified in all populations as expected; PHT was positively correlated with FLH and EHT as well (Figure 1, 2 and 3).

All traits correlation estimates in $F_{3:4}$/Tx714 hybrid yield trials were plotted (Figure 4, 5 and 6). It was found that grain yield was positively with plant height, flag leaf height and ear height and it was negatively correlated with flowering time and grain moisture at early planted tests and late planted tests in the Ki3/NC356 population (Figure 4). In a previous study, Barrero Farfan et al. (2013) also found that plant height, ear height, plant population and test weight were positively correlated with grain yield in commercial temperate hybrids with stronger effects observed in the rest of Texas (which includes College Station) than in the Texas High Plains, and these were still much stronger than those typically observed in the Midwestern US. Therefore, a hypothesis was proposed that taller plants in Ki3/NC356 population would be correlated with higher grain yield in some Texas environments.

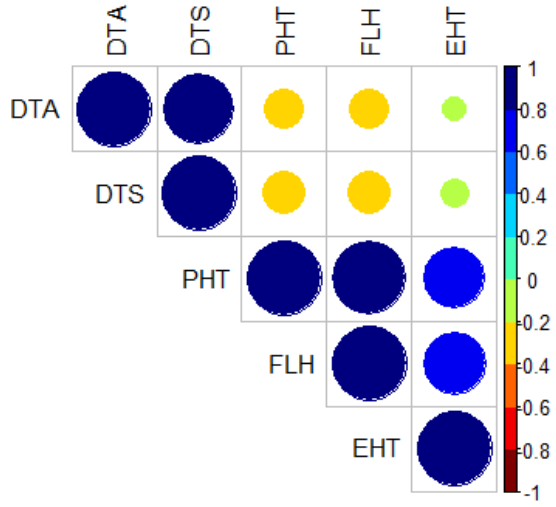

Figure 1 Heat map for traits correlation estimates in $F_{3:4}$ progenies of Ki3/NC356.

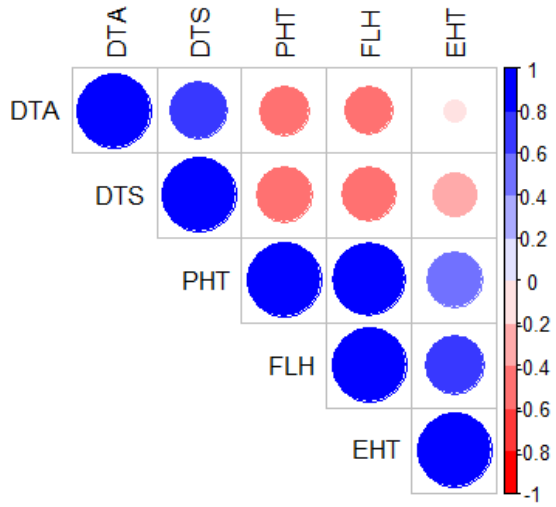

Figure 2 Heat map for traits correlation estimates in $F_{3:4}$ progenies of Tx740/NC356.

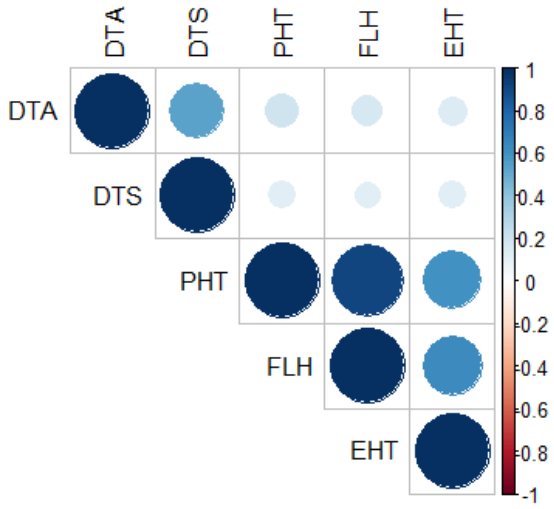

Figure 3 Heat map for traits correlation estimates in $F_{3:4}$ progenies of LH82/LAMA-YC.

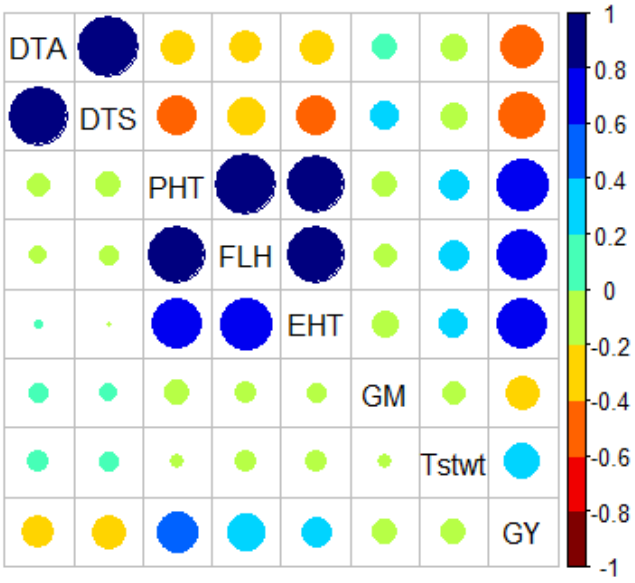

Figure 4 Heat map for traits correlation estimates in $F_{3:4}$/Tx714 hybrids of Ki3/NC356. The lower half diagonal correspond to early planted tests; the upper half diagonal correspond to late planted tests.

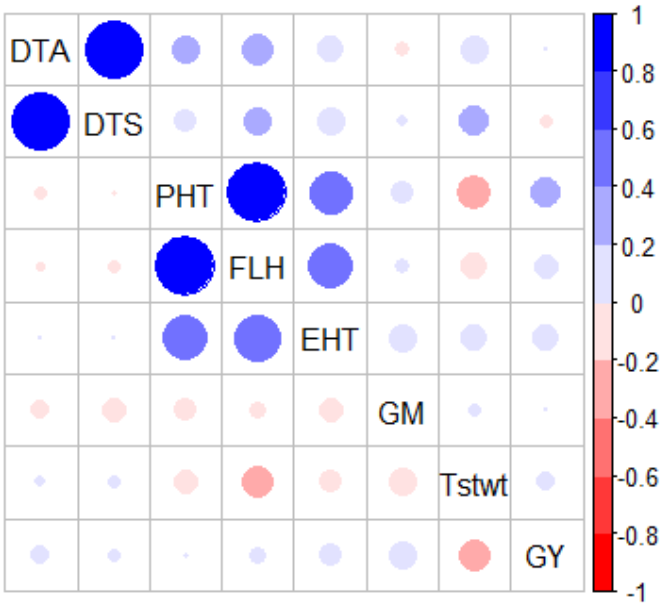

Figure 5 Heat map of traits correlation estimates in $F_{3:4}$/Tx714 hybrids of Tx740/NC356. The lower half diagonal correspond to early planted tests; the upper half diagonal correspond to late planted tests.

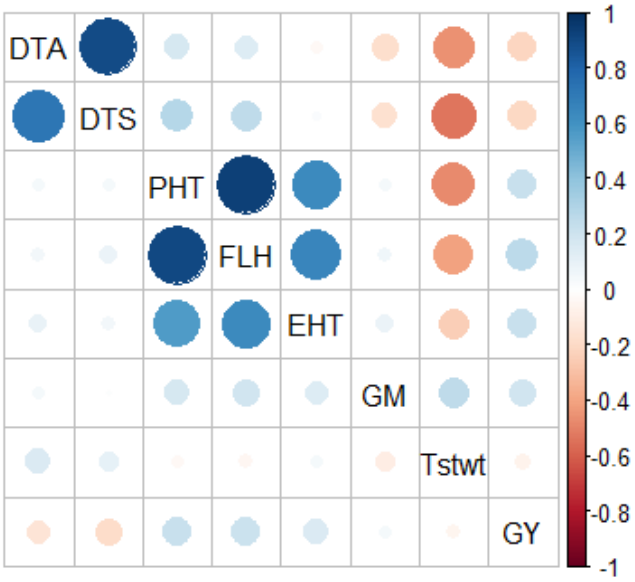

Figure 6 Heat map of traits correlation estimates in $F_{3:4}$/Tx714 hybrids of LH82/LAMA-YC. The lower half diagonal correspond to early planted tests; the upper half diagonal correspond to late planted tests.

Variance components analysis

By partitioning total phenotypic variation following a linear random model (Eq. 1), it was observed in $F_{3:4}$ inbred trials of Ki3/NC356 population that around 50% of total variations for flowering time (DTA and DTS) was explained by the pedigree (i.e. genotype); for PHT, FLH and EHT, the variations from replication, row and random error accounted for over 50% of the total variation (Figure 7). In the $F_{3:4}$ inbred trials of Tx740/NC356 population, the percentage of genetic variation (pedigree) for DTA and EHT were relative high compared with other traits (DTS, PHT and FLH) (Figure 8). In

$F_{3:4}$ inbred trials of LH82/LAMA-YC population, it was noticeable that most variation of DTA and DTS was caused by residual error (Figure 9).

In $F_{3:4}$/Tx714 hybrid yield trials of all three populations, more unexplained error variation than typical was observed across traits, almost exclusively due to excessive rainfall in May and June, 2015 (Figure 10, 11 and 12).

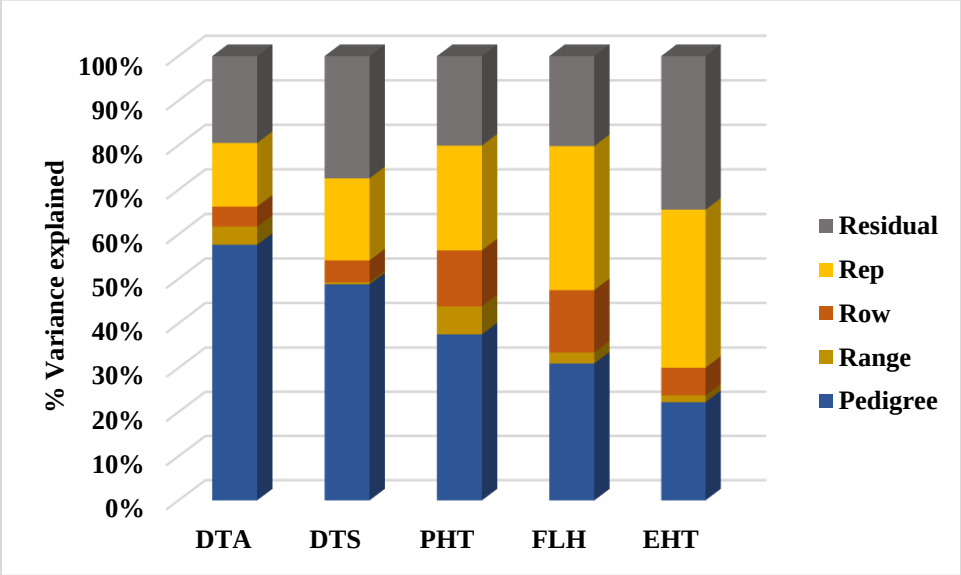

Figure 7 The percentage of total variance explained by each variance component for each trait in $F_{3:4}$ inbred trials of the Ki3/NC356 population.

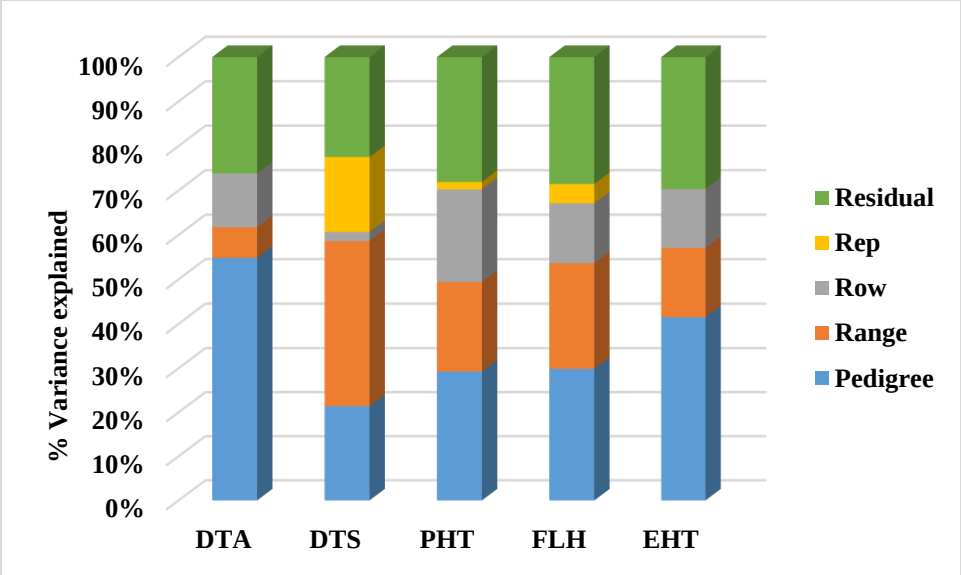

Figure 8 The percentage of total variance explained by each variance component for each trait in $F_{3:4}$ inbred trials of the Tx740/NC356 population.

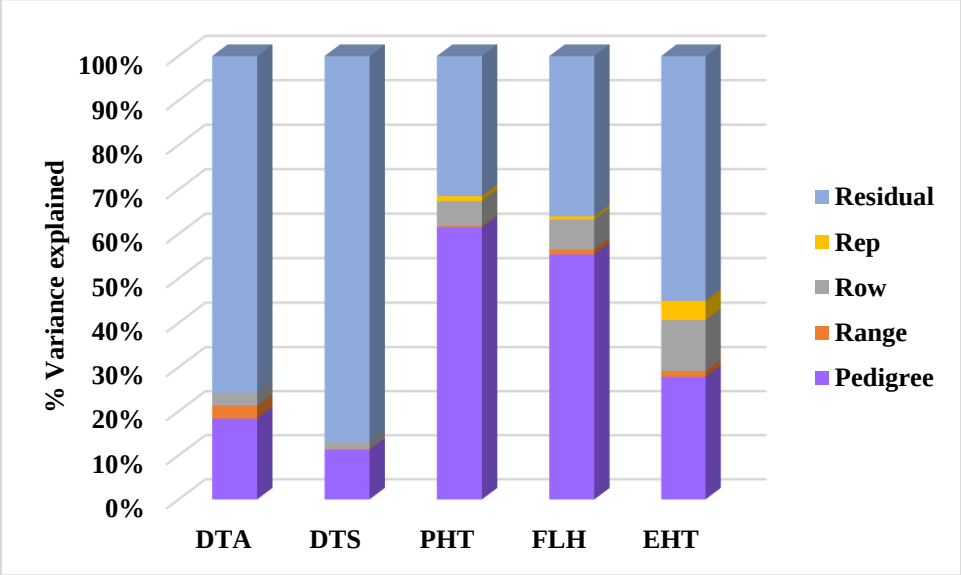

Figure 9 The percentage of total variance explained by each variance component for each trait in $F_{3:4}$ inbred trials of the LH82/LAMA-YC population.

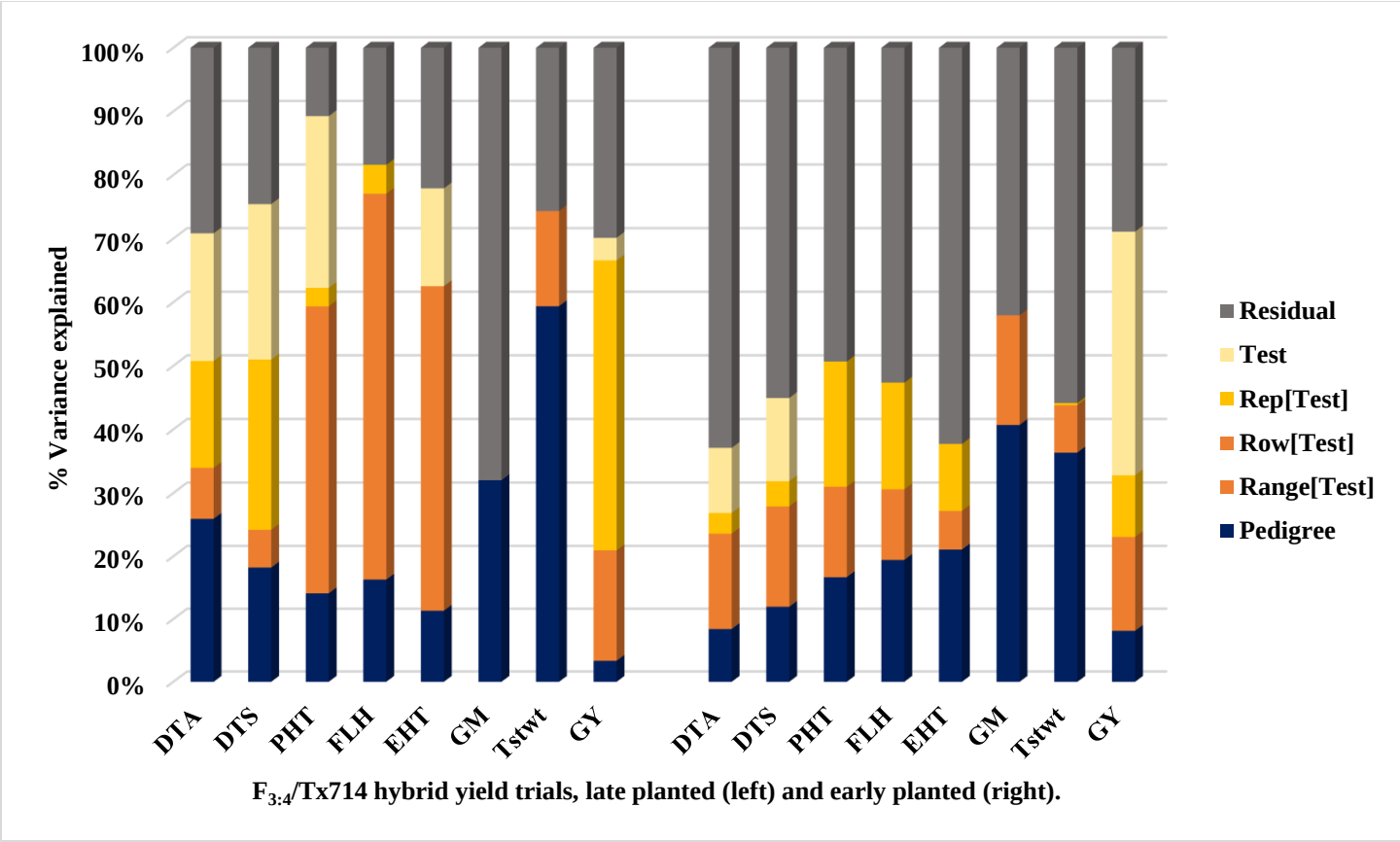

Figure 10 The percentage of total variance explained by each variance component for each trait in $F_{3:4}$/Tx714 hybrid yield trials of the Ki3/NC356 population. "Test" refers to 1, 2 or 4 replicate tests within the planting date treatment.

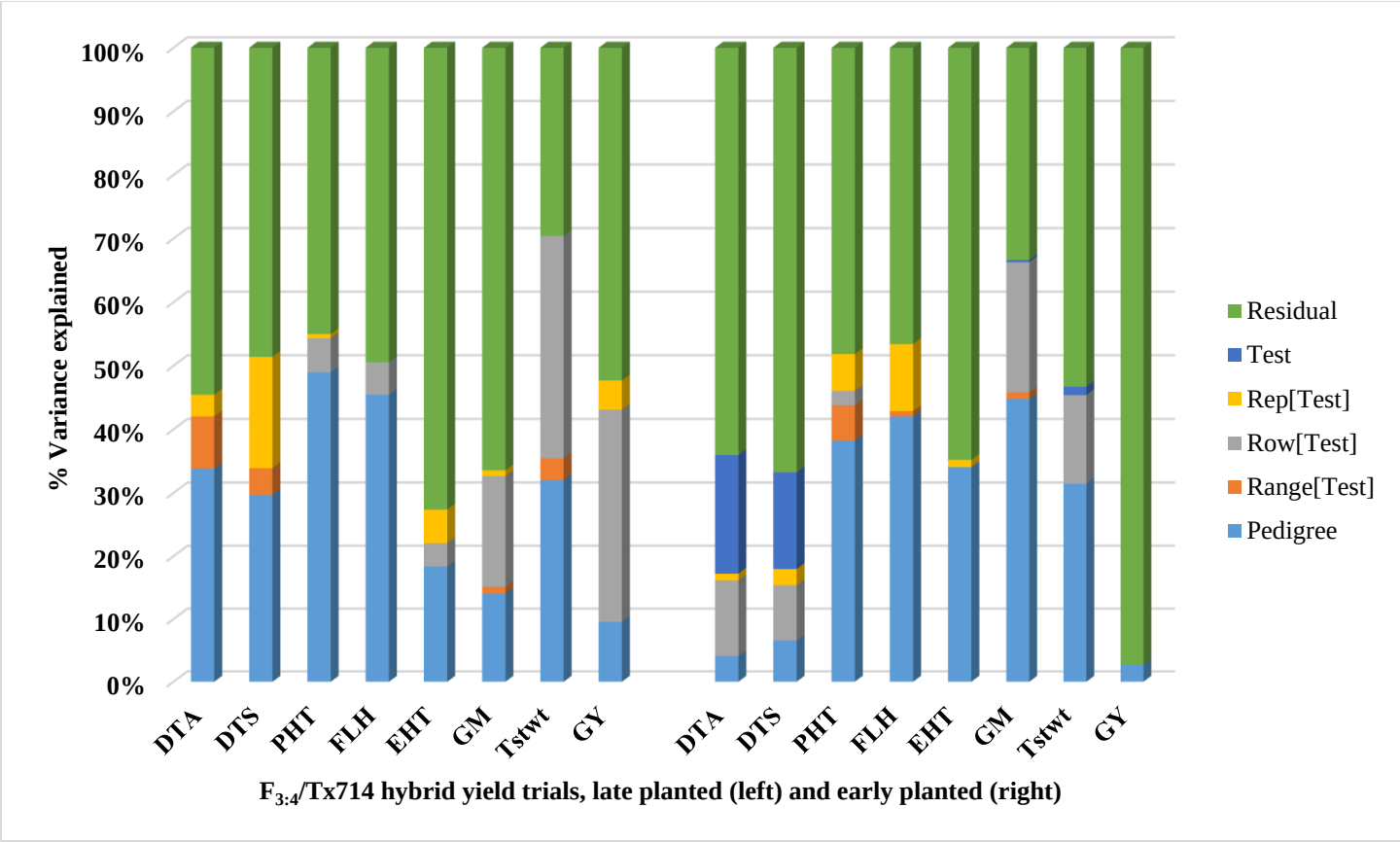

Figure 11 The percentage of total variance explained by each variance component for each trait in $F_{3:4}$/Tx714 hybrid yield trials of the Tx740/NC356 population. "Test" refers to 1, 2 or 4 replicate tests within the planting date treatment.

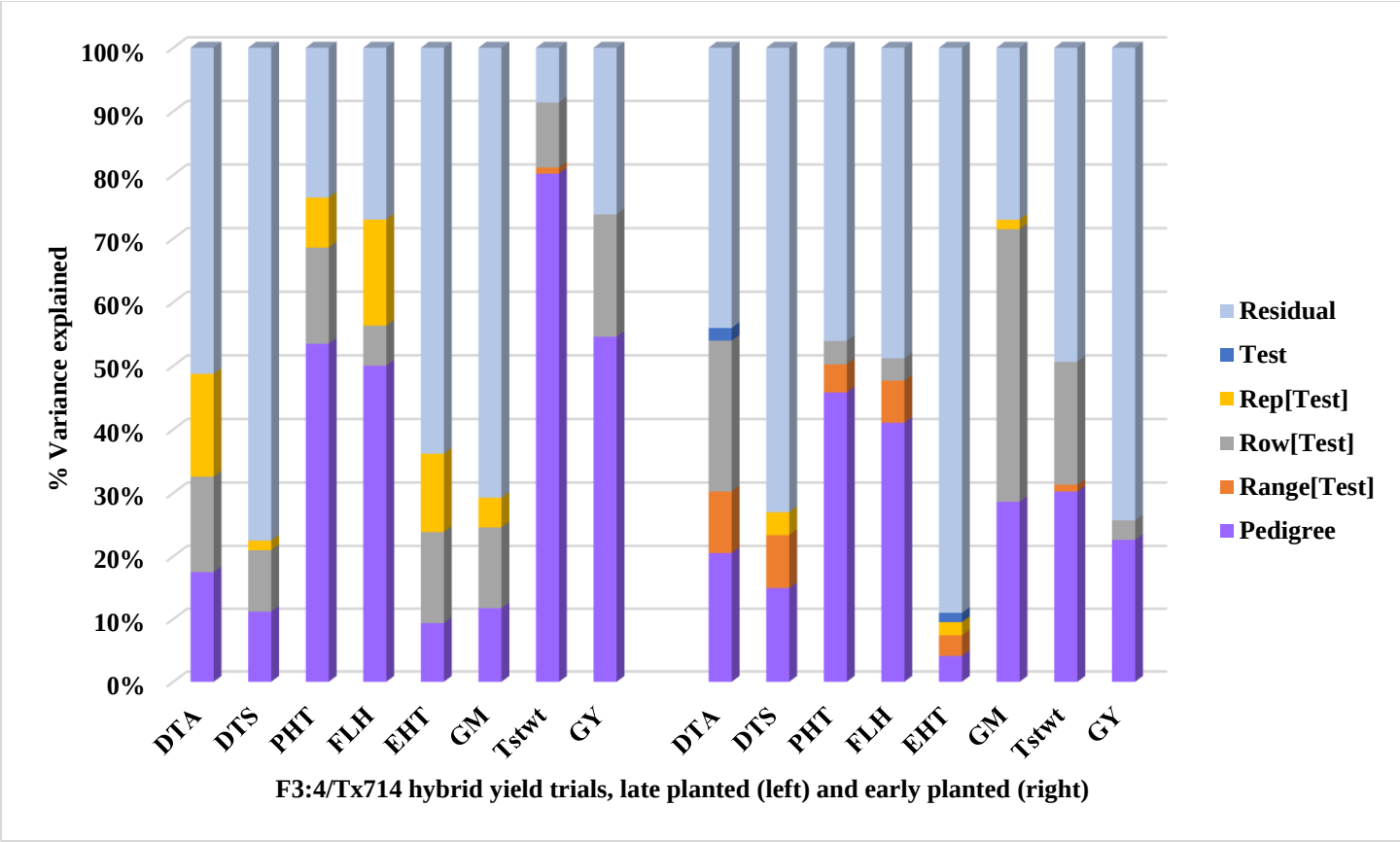

Figure 12 The percentage of total variance explained by each variance component for each trait in $F_{3:4}$/Tx714 hybrid yield trials of the LH82/LAMA-YC population. "Test" refers to 1, 2 or 4 replicate tests within the planting date treatment.

High- density genetic map construction

For population (1) derived from the Ki3/NC356 cross, a subset of 174 $F_{3:4}$ progeny were used for genetic map construction. A genetic map consisting of 1,631 SNP markers in unique bins was constructed with 3,383 polymorphic SNP markers on 10 chromosomes between Ki3 and NC356, the average interval distance was 0.99 cM (Table 23). For the population (2) derived from the Tx740/NC356 cross, a subpopulation of 87 $F_{3:4}$ progeny were used to generate the genetic map, which consisted of 1,438 bin markers with 5,073 polymorphic SNP markers across10 chromosomes. The total map length was 1872.52 cM with 1.3 cM average interval length (Table 24). For the population (3) derived from the LH82/LAMA-YC cross, there were 150 $F_{3:4}$ progeny and 4,297 polymorphic SNP markers used for genetic map construction. The total length of whole genome was 1818.93 cM and the average interval distance was 1.05 cM (Table 25).

Table 25. Summary of the genetic map constructed using $F_{3:4}$ progenies from Ki3/NC356 cross.

Chromosome	No. of bin markers	Length (cM)	Average interval (cM)
1	332	275.98	0.83
2	189	190.48	1.01
3	196	154.18	0.79
4	128	187.41	1.46
5	125	103.22	0.83
6	109	135.09	1.24
7	111	136.71	1.23
8	153	140.16	0.92
9	169	152	0.90
10	119	136.85	1.15
Total	1631	1612.08	0.99

Table 26. Summary of the genetic map constructed using $F_{3:4}$ progenies from Tx740/NC356 cross.

Chromosome	Number of bin markers	Length (cM)	Average interval (cM)
1	225	285.97	1.27
2	143	171.86	1.20
3	163	200.29	1.23
4	135	196.78	1.46
5	159	203.99	1.28
6	114	154.94	1.36
7	126	172.66	1.37
8	141	179.92	1.28
9	124	156.77	1.26
10	108	149.34	1.38
Total	1438	1872.52	1.30

Table 27. Summary of the genetic map constructed using $F_{3:4}$ progenies from LH82/LAMA-YC cross.

Chromosome	Number of bin markers	Length (cM)	Average interval (cM)
1	297	268.04	0.90
2	173	211.91	1.22
3	205	211.83	1.03
4	192	167.01	0.87
5	184	184.04	1.00
6	124	138.99	1.12
7	163	170.41	1.05
8	163	176.77	1.08
9	130	148.56	1.14
10	97	141.37	1.46
Total	1728	1818.93	1.05

The genetic map is an important prerequisite for QTL linkage mapping and very high density genetic maps are beneficial for mapping QTLs and cloning genes. These high density maps are only beginning to be reported in the literature. In maize, Zhou et al. (2016) constructed a ultra- high density genetic map in a set of 314 RILs, the total length of which was 1545.65cM and the average interval distance was 0.37cM; subsequently, a known gene, pericarp color 1 (*P1*), with a high LOD value of 80.78 on chromosome 1 was mapped to verify the quality and accuracy of their genetic map. Chen et al. (2014) constructed an ultra-high-density linkage map for a large set of 708 F_2 maize lines to detect QTLs for tassel branch number, kernel row number and ear length efficiently and also identified one cloned gene *colored* (*r1*) with a high LOD score of 81. Compared to those reported high-density genetic studies, the three genetic maps

generated in the present study covered a similar genome size with average interval distance around 1cM (Figure 7). The higher resolution genetic maps also reduced the QTL support intervals (i.e. narrowed the genetic regions) correlated with the phenotypic variations of each trait and reducing the number of implicated candidate genes.

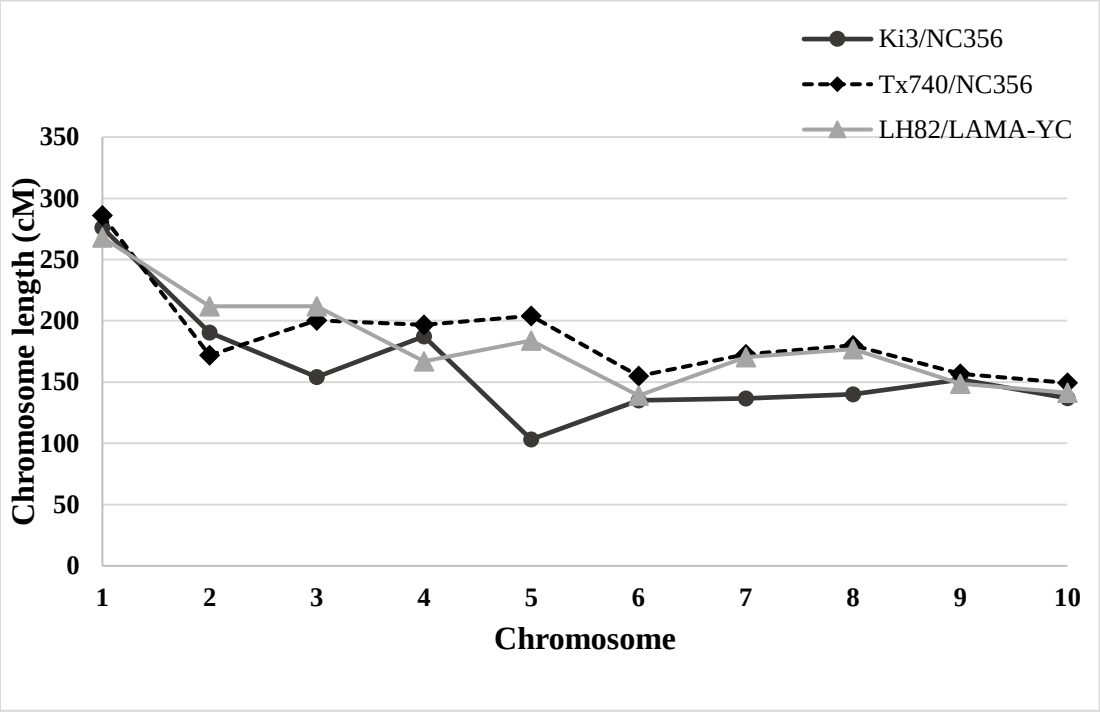

Figure 13 Comparison of three high-density genetic maps.

QTL detection

Most crop QTL linkage mapping studies have used bi-parents populations (i.e. RILs) and estimated QTL effects based on the average effect across each individual lines per se within the population. However, from a breeding perspective, it is more useful to test for QTL effects in a relevant hybrid background. In this study, QTL mapping was conducted separately in $F_{3:4}$ progeny lines per se in addition to the derived testcross hybrids with inbred line Tx714 (Betrán et al. 2004), which is a southern adapted version of, and 98% identical to, the key stiff stalk line B73 (Romay et al. 2013). Tx714 was also used as a tester in a previous association mapping study (Farfan et al. 2015). For each trait, two sets of phenotypic values (BLUEs and BLUPs) accounting for yield spatial variation were used as input. QTL were named based on the trait (i.e. DA stands for days to anthesis), and chromosome, when there were two or more QTL on the same chromosome a decimal designator was used.

QTL detected in population (1) derived from the Ki3/NC356 cross

A total of eighteen QTLs were detected in $F_{3:4}$ lines per se progeny of the Ki3/NC356 population and the corresponding testcross hybrids using BLUEs (Table 26 and Table 27). Three QTLs on chromosome 2, 5 and 10 (*qDA2, qDA5 and qDA10*) collectively explained 40.2% phenotypic variation for days to anthesis. For days to silk, two QTLs on chromosome 2 and 5 (*qDS2* and *qDS5*) explained 16.5% and 10.4% of phenotypic variation respectively and they were at the same positions as *qDA2 and qDA5* (Figure 14), which indicated these two loci with pleiotropic effects on DTA and

DTS. One additional QTL on chromosome 10 (*qDS10*) explained 10.8% of phenotypic variation for days to silk. The additive effects of these QTLs for flowering time (DTA and DTS) ranged from 0.6 to 1.1 days. In this study, it was interesting to find that both *qDA10* and *qDS10* which explained a high percentage phenotypic variation, were located at bin 10.04. Previous studies identified there was a major effect QTL associated with flowering time, photoperiod sensitivity and plant height in tropic maize (Ribaut et al. 1996; Wang et al. 2008) and as reported, bin 10.04 exhibited a more extensive signal for positive selection than other known regions in maize genome, indicating this region was essential for maize adaption(Tian, Stevens, and Buckler 2009). For plant height, two QTLs were identified on chromosome 2 and 8 (*qPH2* and *qPH8*) explaining 16.2 and 18.4% of phenotypic variation respectively and the additive effects were 5.6cm and 6.1cm. For flag leaf height, five QTLs on chromosome 2, 3, 8 and 9 (*qFH2-1, qFH2-2, qFH3, qFH8 and qFH9*) were detected, which explained 49.4% of phenotypic variation and the additive effects ranged from 1.3cm to 4.6cm.

There were only two QTLs identified in the early planted $F_{3:4}$/Tx714 hybrid yield trial: one was on chromosome 9 (*qPH9*) and explained 11.2% of phenotypic variation for plant height with additive affect was 3.2cm; another one was on chromosome 8 (*qGM8*), which explained 11.8% of phenotypic variation for grain moisture. Three QTLs were identified on chromosome 1 and 3 in the late planted $F_{3:4}$/Tx714 hybrid trial: one QTL on chromosome 1 (*qFH1*) explained 23.8% of phenotypic variation for flag leaf height; two QTLs on chromosome 3 (*qTW3* and *qGY3*) significantly affected grain test weight and grain yield, explaining 29.1% and 19.9% of phenotypic variation separately.

An additional four QTLs were detected when using BLUPs of each trait and there were total 22 QTLs detected in $F_{3:4}$ inbred trials and the deriving testcross hybrid yield trails. QTLs found using both BLUEs and BLUPs tended to have larger effects and are also more likely to be real. In brief, these consistent loci included the two QTLs on chromosome 2 and 8 for plant height, three QTLs on chromosome 2, 5 and 10 for flowering time (DTA and DTS), and three QTLs on chromosome 1, 7 and 8 for ear height (Table A3).

QTL detected in population (2) derived from the Tx740/NC356 cross

In total, there were twenty five significant QTLs identified across all traits of the Tx740/NC356 population using BLUE values (Table 28 and Table 29). Six QTLs on chromosome 1, 3, 7, 9 and 10 were detected influencing DTA in $F_{3:4}$ lines per se, which totally explained 76.2% of the total phenotypic variation. Two QTLs (*qDS2* and *qDS3*) were detected controlling days to silk in $F_{3:4}$ progenies and explained 41.9% of the total phenotypic variation. *qDS3* and *qDA3* overlapped at marker Agr_14093 on chromosome 3, which is likely a pleiotropic effect on days to anthesis and days to silk (Figure15). For plant height, there was only one QTL (*qPH8*) identified in the early planted $F_{3:4}$/Tx714 hybrids test; it explained 25% of phenotypic variation. For flag leaf height, five QTLs were identified in $F_{3:4}$ progenies, explaining 63.8% of phenotypic variation and three QTLs were found in testcross hybrids, explaining 86.1% of phenotypic variation. For ear height, *qEH8* was the only QTL identified explaining 28% of phenotypic variation in $F_{3:4}$ progenies. For yield traits, five QTLs were detected for grain test weight in the late planted $F_{3:4}$/Tx714 hybrid yield trials, which collectively explained 87.4% of phenotypic

variation; two QTLs were found in the early planted testcross hybrid yield trials, explaining 29.6% of phenotypic variation for grain yield.

Many of these same QTLs including *qDA9-2*, *qDS2*, *qDS3*, *qEH8* and *qFH10* were all confirmed using BLUPs and explained a high percentage of phenotypic variation. Additionally, unlike population (1), there were additional novel QTLs identified for days to anthesis and grain moisture in $F_{3:4}$/Tx714 hybrid tests by using BLUP values (Table A4).

QTL detected in population (3) derived from the LH82/LAMA-YC cross

Seven QTLs were identified in $F_{3:4}$ progenies and their corresponding $F_{3:4}$/Tx714 hybrids (Tables 30 and 31). Only one QTL was detected for DTA from $F_{3:4}$ progenies on chromosome 8 (*qDA8),* which explained 29.9% phenotypic variation; one region on chromome1 (flanking markers: AgR_10766~ AgR_00859) which appeared to have pleiotropic effects on plant height and flag leaf height (*qPH1-1* and *qFH1*), explaining 12.4% and 12.9% phenotypic variations respectively. In $F_{3:4}$/Tx714 hybrids, two QTLs on chromosome 1 and 8 (*qPH1-2* and *qPH8-2*) influenced plant height, which combined explained 32.5% of phenotypic variation.

Farfan et al. (2015) identified QTV2 (chromosome 7, 164,955,163 bp, Maize GenRef v2.), having a pleiotropic effect on multiple traits (i.e. plant height, days to anthesis and days to silk). In this study, the QTL (*qDS7*) was detected for days to silk by using BLUPs, explaining 6.8% of phenotypic variation and this location overlapped QTV2 from Farfan et al. (2015) which also significantly influenced DTS (Table A5).

Consistent QTL and candidate gene prediction

In the NAM population, Buckler et al. (2009) observed that all DA and DS QTLs had correlated effects on days to silk and days to anthesis, which suggested that the same set of genes involving male and female flowering in maize. In this study there were several QTLs identified with pleiotropic effects on flowering time (Figure 14 and Figure 15).

Despite being grown in the same environments, different QTLs were detected across the three populations as expected for separate diverse bi-parental linkage populations in maize (Holland 2007). Yet, several QTLs were detected consistently at the same locations or tightly linked by using both BLUPs and BLUEs of each line within cross Ki3/NC356 and Tx740/NC356, respectively (Table A6 and Table A7); less surprising, because they share a common parent NC36.

The maize gene annotation database at MaizeGDB (http://maizegdb.org/) provided a number of candidate genes with known functions were predicted for those consistent QTLs verified by BLUEs and BLUPs in Ki3/NC356 population and Tx740/NC356 population (Table 34 and Table 35). Among these putative genes, GRMZM2G367326 located at *qDS10* in Ki3/NC356 population might be the best target for further investigation (Table 34). Its ortholog in *Sorghum bicolor* (Sb06g018510) encodes cryptochrome 2, which was reported to be responsible for blue light recognition and played a role in regulation of flowering time in *Arabidopsis thaliana* and *Oryza sativa* (Guo et al. 1998; Hirose et al. 2006).

Unfortunately but unsurprisingly, comparing QTLs mapped in $F_{3:4}$ progeny and their $F_{3:4}$/Tx714 testcross hybrids, there were no shared QTL. Similar finding have been made by others, this finding clearly demonstrates that QTL mapping in inbred lines per se is irrelevant for progress in plant breeding of crops grown commercially as hybrids. In the early generation of each population, there were still around 25% progenies segregating. The alleles from the tester would likely hamper the QTLs detection accounting for complicated epistasis interactions, which were not considered here.

Conclusion

In this study, grain yield was positively correlated with plant height, flag leaf height and ear height in $F_{3:4}$/Tx714 hybrids yield trials of Ki3/NC356, which indicated selecting taller plants in this population has the potential to improve yield under Southern US conditions. This supports the same finding in commercial hybrid across Southern locations (Barrero Farfan et al. 2013). The other two populations did not clearly show this trend, likely in large part due to the high error of yield in the field.

By using BLUEs and BLUPs for genetic analysis in each linkage population, some large effect QTLs were detected consistently across two different dataset in two populations (1) the Ki3/NC356 population and (2) the Tx740/NC356 population. Several QTLs with pleiotropic effects were detected for flowering time (DTA and DTS). Comparing all the QTLs in each population, even though these three populations shared partial common genetic background, there was no consistent QTLs across all three population.

Table 28. QTLs identified for each trait in Ki3/NC356 $F_{3:4}$ lines per se progeny field test using best linear unbiased estimates.

Trait	QTL	Chr.	LOD	PVE(%)	Add.	Dom.	Left marker	Right marker	LeftPos (bp)	RightPos (bp)
Days to anthesis (days)										
	qDA2	2	6.4	12.0	0.8	0.0	AgR_07178	AgR_07801	17,477,370	17,904,412
	qDA5	5	5.5	9.8	-0.6	-0.5	AgR_15851	AgR_08938	154,808,874	158,543,752
	qDA10	10	9.6	18.4	0.9	-0.4	AgR_17118	AgR_07061	114,621,731	115,147,697
Days to silk (days)										
	qDS2	2	9.9	16.5	1.1	-0.2	AgR_07178	AgR_07801	17,477,370	17,904,412
	qDS5	5	6.5	10.4	-0.9	-0.2	AgR_08938	AgR_15851	154,808,874	158,543,752
	qDS10	10	7.0	10.8	0.8	-0.7	AgR_13720	AgR_03430	121,287,106	121,725,750
Plant height (cm)										
	qPH2	2	10.4	16.2	5.6	1.8	AgR_14588	AgR_02312	37,133,343	37,446,316
	qPH8	8	11.7	18.4	6.1	1.6	AgR_03251	AgR_16454	-	17,383,010
Flag leaf height (cm)										
	qFH2-1	2	5.6	7.9	-1.3	5.9	AgR_02478	AgR_07794	-	12,066,900
	qFH2-2	2	9.1	13.0	4.2	1.9	AgR_07808	AgR_17305	19,989,478	20,205,866
	qFH3	3	5.4	7.5	3.3	0.3	AgR_02541	AgR_14919	12,133,117	13,273,221
	qFH8	8	10.3	15.1	4.6	1.4	AgR_06260	AgR_06262	10,760,848	11,664,379
	qFH9	9	4.5	6.0	2.8	-1.6	AgR_13310	AgR_06594	10,093,838	10,806,295

LOD: log of odds ratio for QTL; PVE(%): the phenotypic variation explained by individual QTL; Add: the additive effect value; Dom: the dominance effect value.

Chr: chromosome; LeftPos and RightPos: the physical position of left and right markers according to Maize B73_RefGen_v2.

"-": SNP information is non-available.

Table 29. QTLs identified for each trait in Ki3/NC356 $F_{3:4}$ /Tx714 hybrid yield trials using best linear unbiased estimates.

Trait	QTL	Chr.	LOD	PVE(%)	Add.	Left marker	Right marker	LeftPos (bp)	RightPos (bp)	Test
Plant height (cm)										
	qPH9	9	4.1	11.2	3.2	AgR_16871	AgR_13440	103,875,951	103,881,289	Early planted
Flag leaf height (cm)										
	qFH1	1	5.0	23.8	-5.9	AgR_10297	AgR_03528	7,866,418	8,100,477	Late planted
Grain moisture (%)										
	qGM8	8	4.5	11.8	-0.5	AgR_13271	AgR_13268	164,812,412	164,040,056	Early planted
Grain testweight (kg/hL)										
	qTW3	3	5.5	29.1	-0.8	AgR_14904	AgR_11283	6,433,396	6,937,795	Late planted
Grain yield (ton/ha)										
	qGY3	3	4.9	19.9	-0.5	AgR_15228	AgR_15229	222,451,007	222,907,159	Late planted

LOD: log of odds ratio for QTL; PVE(%): the phenotypic variation explained by individual QTL; Add: the additive effect value.
Chr: chromosome; LeftPos and RightPos: the physical position of left and right markers according to Maize B73_RefGen_v2

Table 30. QTLs identified for each trait in Tx740/NC356 $F_{3:4}$ lines per se progeny field test using best linear unbiased estimates.

Trait	QTL	Chr.	LOD	PVE(%)	Add.	Dom.	Left marker	Right marker	LeftPos (bp)	RightPos (bp)
Days to anthesis (days)										
	qDA1	1	4.3	7.8	0.0	-1.0	AgR_10336	AgR_14085	27,980,406	29,355,902
	qDA3	3	4.5	8.6	-0.6	0.1	AgR_14900	AgR_14903	4,704,029	5,589,411
	qDA7	7	6.1	11.7	0.6	-0.2	AgR_06163	AgR_09424	141,427,513	142,726,091
	qDA9-1	9	7.6	15.2	-0.1	1.2	AgR_09728	AgR_16751	11,501,782	12,023,097
	qDA9-2	9	9.2	18.5	-0.8	-0.4	AgR_16916	AgR_16919	136,785,821	137,787,366
	qDA10	10	7.0	14.4	0.1	-1.2	AgR_13756	AgR_13761	138,832,523	140,049,011
Days to silk (days)										
	qDS2	2	5.1	20.5	1.3	-0.6	AgR_04201	AgR_14654	63,819,796	68,700,035
	qDS3	3	5.3	21.5	-1.4	0.6	AgR_14903	AgR_11281	5,589,411	5,853,098
Flag leaf height (cm)										
	qFH1	1	8.5	14.8	5.0	0.2	AgR_01316	AgR_07420	-	34,994,417
	qFH3-1	3	4.8	7.3	0.3	5.7	AgR_14976	AgR_14977	54,121,561	54,475,038
	qFH3-2	3	6.6	10.6	0.6	8.0	AgR_11459	AgR_15058	134,772,775	135,440,139
	qFH7	7	5.5	9.1	-0.7	7.0	AgR_03161	AgR_16386	159,134,313	159,905,219
	qFH9	9	11.6	21.9	6.3	1.2	AgR_06839	AgR_06842	145,845,214	146,154,984
Ear height (cm)										
	qEH8	8	6.3	28.0	-3.9	1.2	AgR_16586	AgR_09612	113,658,795	117,761,504

LOD: log of odds ratio for QTL; PVE(%): the phenotypic variation explained by individual QTL; Add: the additive effect value; Dom: the dominance effect value.

Chr: chromosome; LeftPos and RightPos: the physical position of left and right markers according to Maize B73_RefGen_v2.

"-": SNP information is non-available.

Table 31. QTLs identified for each trait in Tx740/NC356 $F_{3:4}$/Tx714 hybrid yield trials using best linear unbiased estimates.

Trait	QTL	Chr.	LOD	PVE(%)	Add	Left marker	Right marker	LeftPos (bp)	RightPos (bp)	Test
Plant height (cm)										
	qPH8	8	4.5	25.0	-4.0	AgR_06450	AgR_03232	131,179,631	131,516,951	Early planted
Flag leaf height (cm)										
	qFH5	5	5.1	30.5	4.3	AgR_15677	AgR_05357	20,774,070	22,630,187	Early planted
	qFH3-3	3	4.5	22.3	4.1	AgR_08334	AgR_02606	173,014,257	173,803,352	Late planted
	qFH10	10	6.0	33.2	-0.4	AgR_13739	AgR_10061	131,043,066	132,512,811	Late planted
Grain testweight (kg/hL)										
	qGW2	2	6.3	8.7	0.3	AgR_11144	AgR_04333	177,751,572	178,977,489	Late planted
	qGW3	3	5.8	8.0	0.0	AgR_11560	AgR_08356	184,359,096	185,242,791	Late planted
	qGW4	4	8.2	12.9	0.0	AgR_15381	AgR_08591	81,595,192	143,716,879	Late planted
	qGW5	5	15.2	38.8	0.8	AgR_15671	AgR_02770	18,133,730	18,770,133	Late planted
	qGW7	7	10.2	18.9	0.0	AgR_10111	AgR_00900	170,032,283	-	Late planted
Grain yield (ton/ha)										
	qGY5	5	4.7	11.3	-0.4	AgR_08984	AgR_00280	181,014,434	-	Early planted
	qGY7	7	7.1	18.3	-0.6	AgR_00754	AgR_05976	-	8,687,081	Early planted

LOD: log of odds ratio for QTL; PVE(%): the phenotypic variation explained by individual QTL; Add: the additive effect value.
Chr: chromosome; LeftPos and RightPos: the physical position of left and right markers according to Maize B73_RefGen_v2.
"-": SNP information is non-available.

Table 32. QTLs identified for each trait in LH82/LAMA-YC $F_{3:4}$ lines per se progeny field test using best linear unbiased estimates.

Trait	QTL	Chr.	LOD	PVE(%)	Add.	Dom.	Left marker	Right marker	LeftPos (bp)	RightPos (bp)
Days to anthesis (days)										
	qDA8	8	13.7	29.9	-0.4	-0.2	AgR_06430	AgR_13177	122,901,209	124,357,232
Plant height (cm)										
	qPH1-1	1	5.3	12.4	-4.9	0.6	AgR_10766	AgR_00859	278,195,980	-
	qPH8-1	8	6.0	14.1	-5.4	0.8	AgR_13177	AgR_16610	124,357,232	124,845,248
Flag leaf height (cm)										
	qFH1	1	6.0	12.9	-4.0	-0.3	AgR_10766	AgR_00859	278,195,980	-
	qFH4	4	4.1	8.5	3.3	-1.1	AgR_15487	AgR_08648	173,859,864	174,913,568

LOD: log of odds ratio for QTL; PVE(%): the phenotypic variation explained by individual QTL; Add: the additive effect value; Dom: the dominance effect value.
Chr: chromosome; LeftPos and RightPos: the physical position of left and right markers according to Maize B73_RefGen_v2.
"-": SNP information is non-available.

Table 33. QTLs identified for each trait in LH82/LAMA-YC $F_{3:4}$/Tx714 hybrid yield trials using best linear unbiased estimates.

Trait	QTL	Chr.	LOD	PVE(%)	Add.	Left marker	Right marker	LeftPos (bp)	RightPos (bp)	Test
Plant height (cm)										
	qPH1-2	1	4.2	16.8	-0.8	AgR_14073	AgR_03564	21,074,756	23,965,965	Early planted
	qPH8-2	8	4.1	15.7	-3.9	AgR_06455	AgR_13199	132,854,286	133,439,049	Early planted

LOD: log of odds ratio for QTL. PVE(%): the phenotypic variation explained by individual QTL; Add.: the additive effect value.
Chr.:chromosome; LeftPos and RightPos: the physical position of left and right markers according to Maize B73_RefGen_v2.

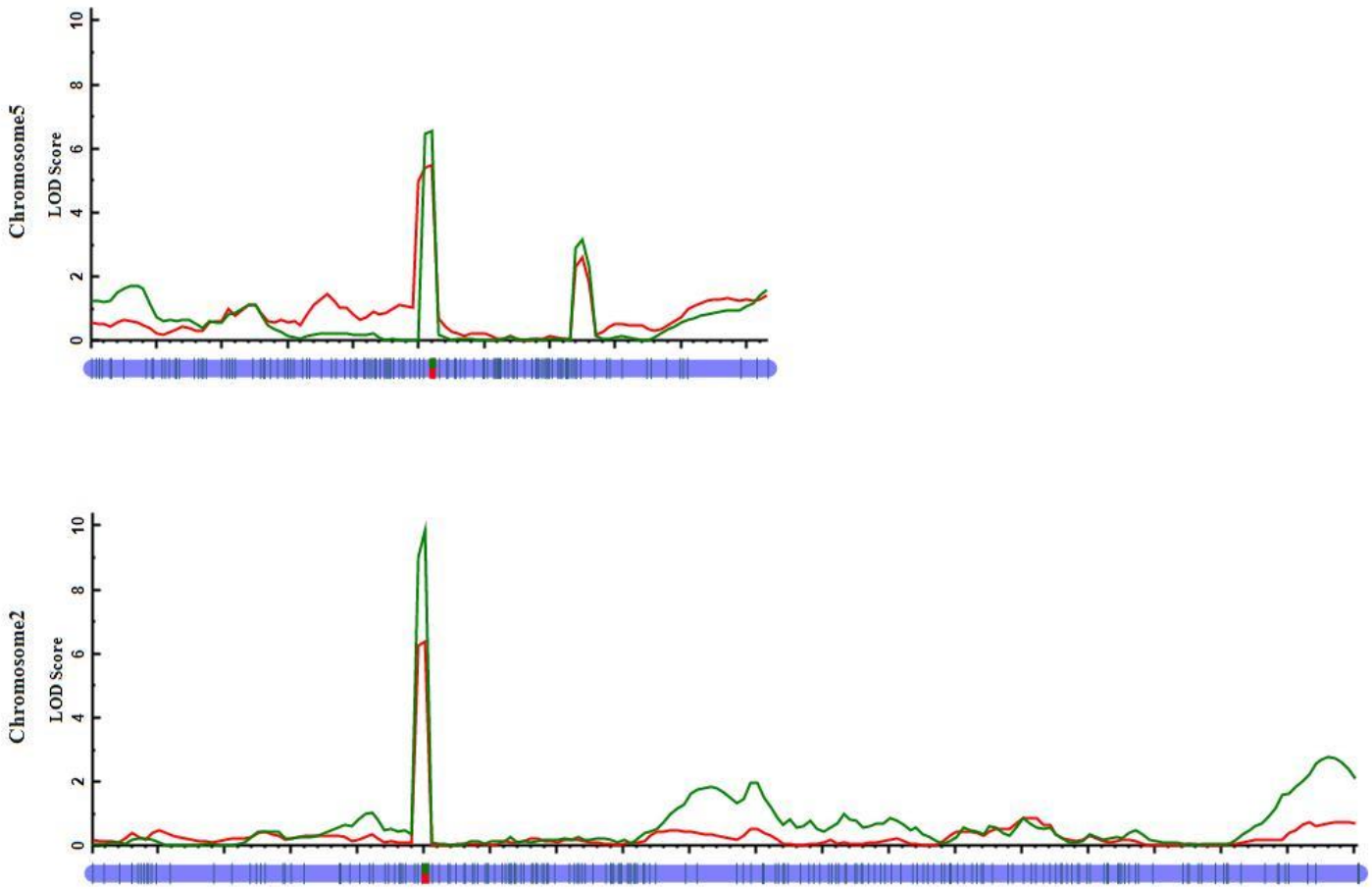

Figure 14 Pleiotropic QTLs for DTA and DTS identified in Ki3/NC356 $F_{3:4}$ lines per se progeny field test using BLUEs. Green color peak was DTS QTL; red color peak was DTA QTL.

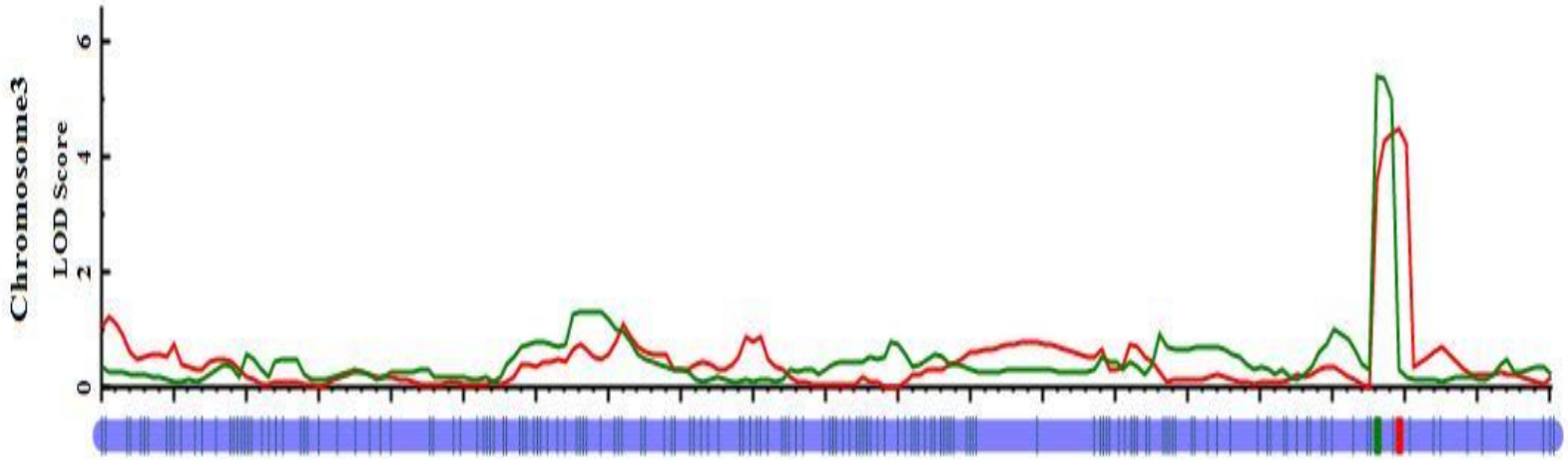

Figure 15 Pleiotropic QTLs for DTA and DTS identified in Tx740/NC356 $F_{3:4}$ lines per se progeny field test using BLUEs. Green color peak was DTS QTL; red color peak was DTA peak.

Table 34. Candidate genes located in consistent QTL intervals of the Ki3/NC356 population.

QTL	Chr.	Gene ID	Annotation
qPH2	2	GRMZM2G135410	MYB-related-transcription factor 93
	2	GRMZM2G163494	Nitrate transporter 4
qFH2-2	2	GRMZM2G028969	AP2-EREBP-transcription factor 185
	2	GRMZM2G105137	MYB-transcription factor 104
	2	GRMZM2G172936	AP2-EREBP-transcription factor 6
qFH3	3	AC233943.1_FG002	ra2 lob domain protein, tassel many-branched, upright, not conical like ra1; irregular kernel placement
	3	GRMZM2G052377	MYB-transcription factor 20
	3	GRMZM2G054559	Phospholipase D1
qTW3	3	GRMZM2G001875	MYB-transcription factor 131
qGM8	8	GRMZM2G133806	ial1 (ig1-as2 like1)
	8	GRMZM2G163081	rpl5b (60S ribosomal protein L5-1 homolog b)
	8	GRMZM2G172001	Alfin-like-transcription factor 8 (alf8)
	8	GRMZM2G172032	Diphosphocytidyl methyl erythritol synthase2 (dmes2)
qDS10	10	GRMZM2G367326	Sorghum bicolor ortholog: Sb06g018510, cryptochrome 2, blue light photoreceptor

Table 35. Candidate genes located in consistent QTL intervals of theTx740/NC356 population.

QTL	Chr.	Gene ID	Annotation
qDS2	2	GRMZM2G145407	ZIM-transcription factor 33
qDS3	3	GRMZM2G397755	bHLH-transcription factor 70
qEH8	8	GRMZM2G055489	Sucrose-phosphatase1
qDA9-2	9	GRMZM2G700011	MYB-related-transcription factor 72
qFH10	10	GRMZM2G040664	AP2-EREBP-transcription factor 86
	10	GRMZM2G122340	Cytokinin oxidase11, gene prodcuts: cytokinin dehydrogenase
	10	GRMZM2G465091	TCP-transcription factor 14

CHAPTER IV

CONCLUSIONS

In summary, three bi-parental linkage populations (Ki3/NC356, Tx740/NC356 and LH82/LAMA-YC) from tropical maize germplasm were used in this study for validating the effects of three QTVs and QTL mapping. By single marker analysis, QTV1 and QTV3 were consistently confirmed as significant for plant height across $F_{3:4}$ inbred and the corresponding testcross hybrid trials in the biggest population Ki3/NC356; and the alleles with positive effects at these two loci were from NC356. In addition, grain yield was positively correlated with plant height in $F_{3:4}$/Tx714 hybrid yield trials, which were in agreement with previous study of Texas AgriLife Corn Performance Trials (Barrero Farfan et al. 2013) and suggested that selecting higher plants in population Ki3/NC356 could improve grain yield. The other two populations did not clearly show this trend, likely in large part due to the high error of yield in the field.

By using BLUEs and BLUPs for QTL mapping in each linkage population, some large effect QTLs were detected consistently across two different dataset in two populations (1) the Ki3/NC356 population and (2) the Tx740/NC356 population. Several QTLs with pleiotropic effects were detected for flowering time (DTA and DTS). Comparing all the QTLs in each population, even though these three populations shared partial common genetic background, there was no consistent QTLs across all three

population. Among putative genes, GRMZM2G367326 located at *qDS10* in Ki3/NC356 population might be the best target for further investigation (Table 34). Its ortholog in *Sorghum bicolor* (Sb06g018510) encodes cryptochrome 2, which was reported to be responsible for blue light recognition and played a role in regulation of flowering time in *Arabidopsis thaliana* and *Oryza sativa* (Guo et al. 1998; Hirose et al. 2006).

It is worthwhile to further investigate all these QTLs detected in these three unique tropical-adaptive maize populations, which would provide profound clues to uncover the genetic and biological mechanisms regulating maize grain yield in Texas and be the promising resources to improve maize production under Texas environments.

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APPENDIX

Table A1. Number of plots laid out in $F_{3:4}$/Tx714 hybrid yield trials for each population.

Pop.	Non-irrigated tests (March 16 th, 2015)		Irrigated tests (March 2nd, 2015)			
	1row 4 reps	1row 2reps	1row 4 reps	1row 2reps	1row 2 reps	1row 1 reps
1	CS15YKD42 224plots	CS15YKD22 148plots	CS15YKW42 232plots	CS15YKW22 150plots	CS15YKW21 114plots	CS15YKW11 27plots
2		CS15YTD22 122plots		CS15YTW22 122plots	CS15YTW21 20plots	CS15YTW11 13plots
3		CS15YLD22 70plots		CS15YLW22 72plots	CS15YLW21 92plots	CS15YLW11 26plots

Table A2. Basic statistics summary of BLUEs and BLUPs for each population**.**

Trait	Test	BLUEs		BLUPs	
		Mean	StdError	Mean	StdError
			Ki3/NC356		
DTA (days)	$F_{3:4}$ inbred	85.5	2.0	85.5	1.7
	$F_{3:4}$/Tx714 early planted	78.1	1.1	78.2	0.2
	$F_{3:4}$/Tx714 late planted	69.5	2.3	70.8	1.0
DTS (days)	$F_{3:4}$ inbred	86.2	2.1	86.2	1.7
	$F_{3:4}$/Tx714 early planted	80.2	1.2	80.5	0.3
	$F_{3:4}$/Tx714 late planted	71.6	2.6	73.2	0.9
PHT (cm)	$F_{3:4}$ inbred	146.4	11.7	147.0	8.6
	$F_{3:4}$/Tx714 early planted	222.4	8.5	222.2	2.6
	$F_{3:4}$/Tx714 late planted	242.3	14.5	234.9	5.4
FLH (cm)	$F_{3:4}$ inbred	114.8	10.1	115.3	7.3
	$F_{3:4}$/Tx714 early planted	182.0	7.6	182.0	2.8
	$F_{3:4}$/Tx714 late planted	191.1	10.5	188.7	5.1
EHT (cm)	$F_{3:4}$ inbred	50.0	7.6	50.7	4.0
	$F_{3:4}$/Tx714 early planted	92.6	8.1	93.4	2.6
	$F_{3:4}$/Tx714 late planted	99.7	7.9	99.0	3.6
GM (%)	$F_{3:4}$/Tx714 early planted	13.2	1.1	13.2	0.6
	$F_{3:4}$/Tx714 late planted	12.1	0.0	12.1	0.0
Tstwt (kg/hL)	$F_{3:4}$/Tx714 early planted	59.1	1.2	59.1	0.6
	$F_{3:4}$/Tx714 late planted	58.0	1.1	57.9	0.8
GY (ton/ha)	$F_{3:4}$/Tx714 early planted	9.4	1.2	9.3	0.3
	$F_{3:4}$/Tx714 late planted	7.2	0.9	7.2	0.1
			Tx740/NC356		
DTA (days)	$F_{3:4}$ inbred	88.5	1.5	88.5	1.2
	$F_{3:4}$/Tx714 early planted	79.2	0.6	79.0	0.1
	$F_{3:4}$/Tx714 late planted	68.3	0.7	68.2	0.3
DTS (days)	$F_{3:4}$ inbred	95.0	2.9	94.8	1.8
	$F_{3:4}$/Tx714 early planted	82.0	0.8	81.7	0.1
	$F_{3:4}$/Tx714 late planted	71.7	0.9	71.7	0.5
PHT (cm)	$F_{3:4}$ inbred	128.9	9.6	129.5	5.9
	$F_{3:4}$/Tx714 early planted	235.5	7.5	234.8	4.0
	$F_{3:4}$/Tx714 late planted	258.4	8.2	258.3	5.3
FLH (cm)	$F_{3:4}$ inbred	96.9	8.9	97.4	5.6

Table A2. Continued

Trait	Test	BLUEs		BLUPs	
		Mean	StdError	Mean	StdError
	$F_{3:4}$/Tx714 early planted	191.3	6.6	190.3	3.7
	$F_{3:4}$/Tx714 late planted	209.9	7.9	209.8	4.9
EHT (cm)	$F_{3:4}$ inbred	43.2	6.7	43.5	4.6
	$F_{3:4}$/Tx714 early planted	99.8	8.4	99.7	4.0
	$F_{3:4}$/Tx714 late planted	109.6	6.9	110.2	2.3
GM (%)	$F_{3:4}$/Tx714 early planted	12.9	0.9	12.9	0.5
	$F_{3:4}$/Tx714 late planted	12.2	0.2	12.2	0.0
Tstwt (kg/hL)	$F_{3:4}$/Tx714 early planted	58.8	1.1	58.8	0.5
	$F_{3:4}$/Tx714 late planted	57.7	1.0	57.6	0.5
GY (ton/ha)	$F_{3:4}$/Tx714 early planted	10.8	0.9	10.8	0.0
	$F_{3:4}$/Tx714 late planted	7.7	0.8	7.6	0.2
		LH82/LAMA-YC			
DTA (days)	$F_{3:4}$ inbred	85.3	0.6	85.2	0.1
	$F_{3:4}$/Tx714 early planted	75.7	0.8	75.6	0.2
	$F_{3:4}$/Tx714 late planted	66.8	1.0	66.7	0.3
DTS (days)	$F_{3:4}$ inbred	86.8	0.5	86.8	0.1
	$F_{3:4}$/Tx714 early planted	78.3	0.7	78.3	0.2
	$F_{3:4}$/Tx714 late planted	69.7	1.1	69.6	0.2
PHT (cm)	$F_{3:4}$ inbred	114.1	12.1	113.8	9.0
	$F_{3:4}$/Tx714 early planted	214.6	9.1	214.0	5.4
	$F_{3:4}$/Tx714 late planted	235.8	10.3	235.5	7.8
FLH (cm)	$F_{3:4}$ inbred	85.7	9.9	85.6	7.1
	$F_{3:4}$/Tx714 early planted	174.6	8.4	174.7	4.6
	$F_{3:4}$/Tx714 late planted	191.9	9.8	191.8	7.3
EHT (cm)	$F_{3:4}$ inbred	30.4	5.8	30.5	2.7
	$F_{3:4}$/Tx714 early planted	88.7	6.3	88.4	0.4
	$F_{3:4}$/Tx714 late planted	100.7	7.6	100.0	1.5
GM (%)	$F_{3:4}$/Tx714 early planted	11.8	0.8	11.8	0.4
	$F_{3:4}$/Tx714 late planted	12.3	0.2	12.3	0.0
Tstwt (kg/hL)	$F_{3:4}$/Tx714 early planted	58.0	1.3	58.0	0.5
	$F_{3:4}$/Tx714 late planted	57.0	1.5	57.0	1.3
GY (ton/ha)	$F_{3:4}$/Tx714 early planted	9.1	1.4	9.4	0.4
	$F_{3:4}$/Tx714 late planted	7.9	1.3	7.9	0.9

StdError: standard error.

Table A3. QTLs mapped in Ki3/NC356 population using best linear unbiased predictions.

Trait	Test	Chr.	Left marker	Right marker	LOD	PVE(%)	Add.	Dom.	LeftPos (bp)	RightPos (bp)
Days to anthesis (days)										
	$F_{3:4}$ inbred	2	AgR_10841	AgR_10236	10.4	14.9	0.8	-0.2	16,547,063	17,477,370
	$F_{3:4}$ inbred	5	AgR_00238	AgR_08930	5.5	7.4	-0.6	0	154,808,874	149,998,432
	$F_{3:4}$ inbred	10	AgR_13728	AgR_10048	11.2	16.7	0.8	0	123,441,081	123,956,269
Days to silk (days)										
	$F_{3:4}$ inbred	2	AgR_01403	AgR_04093	11.5	17.8	0.8	-0.5	-	15,973,209
	$F_{3:4}$ inbred	5	AgR_15851	AgR_08938	7.6	11.4	-0.7	-0.2	158,543,752	154,808,874
	$F_{3:4}$ inbred	10	AgR_13720	AgR_03430	8	12	0.6	-0.5	121,287,106	121,725,750
Plant height (cm)										
	$F_{3:4}$ inbred	2	AgR_14588	AgR_02312	10.5	16.3	4.1	1.2	37,133,343	37,446,316
	$F_{3:4}$ inbred	8	AgR_03251	AgR_16454	11.9	18.5	4.4	1.7	-	17,383,010
Flag leaf height (cm)										
	$F_{3:4}$ inbred	2	AgR_10829	AgR_07792	7.7	6.9	-2.5	0.8	9,019,296	9,971,514
	$F_{3:4}$ inbred	2	AgR_02478	AgR_07794	5.8	5.1	0	3.7	-	12,066,900
	$F_{3:4}$ inbred	2	AgR_17305	AgR_02306	14.6	14.6	3.5	0.6	20,205,866	21,903,459
	$F_{3:4}$ inbred	3	AgR_02541	AgR_14919	7.4	6.7	2.4	-0.2	12,133,117	13,273,221
	$F_{3:4}$ inbred	4	AgR_05117	AgR_08600	7.5	7.3	-2.3	-1.3	157,911,888	150,633,678
	$F_{3:4}$ inbred	8	AgR_06511	AgR_13248	5.6	4.9	0.1	3.6	155,643,006	153,861,116
	$F_{3:4}$ inbred	8	AgR_06262	AgR_06260	14.6	14.3	3.4	0.8	11,664,379	10,760,848
	$F_{3:4}$ inbred	9	AgR_13435	AgR_09825	4.5	3.9	0	3.3	101,902,713	100,924,789

Table A3. Continued

Trait	Test	Chr.	Left marker	Right marker	LOD	PVE(%)	Add.	Dom.	LeftPos (bp)	RightPos (bp)
Ear height (cm)										
	$F_{3:4}$ inbred	1	AgR_07139	AgR_02264	4.3	10.7	-1.5	0.9	4,764,811	6,200,972
	$F_{3:4}$/Tx714 late planted	7	AgR_09285	AgR_01431	6.2	22.6	-0.1	-	3,000,255	-
	$F_{3:4}$/Tx714 late planted	8	AgR_06243	AgR_00059	4.5	15.8	-0.1	-	2,901,121	-
Grain moisture (%)										
	$F_{3:4}$/Tx714 early planted	2	AgR_11066	AgR_14744	4.7	9.4	0.2	-	142,684,984	149,186,389
	$F_{3:4}$/Tx714 early planted	8	AgR_13268	AgR_13271	6.3	12.9	-0.3	-	164,040,056	164,812,412
Grain testweight (kg/hL)										
	$F_{3:4}$/Tx714 late planted	3	AgR_14904	AgR_11283	5.3	28	-0.5	-	6,433,396	6,937,795

LOD: log of odds ratio for QTL; PVE(%): the phenotypic variation explained by individual QTL; Add: the additive effect value; Dom: the dominance effect value.
Chr: chromosome; LeftPos and RightPos: the physical position of left and right markers according to Maize B73_RefGen_v2.
"-":non-available data

Table A4. QTLs mapped in Tx740/NC356 population using best linear unbiased predictions.

Trait	Test	Chr.	Left Marker	Right Marker	LOD	PVE (%)	Add	Dom	LeftPos (bp)	RightPos (bp)
Days to anthesis (days)										
	$F_{3:4}$ inbred	9	AgR_16916	AgR_16919	6.8	25.1	-0.7	-0.1	136,785,821	137,787,366
	$F_{3:4}$/Tx714 late planted	5	AgR_17259	AgR_01811	7.5	25.3	0.0	-	213297503	-
	$F_{3:4}$/Tx714 late planted	9	AgR_00033	AgR_13323	7.5	25.7	-0.2	-	-	17439932
	$F_{3:4}$/Tx714 late planted	9	AgR_16741	AgR_03334	6.0	19.0	0.0	-	7620752	8414792
	$F_{3:4}$/Tx714 early planted	1	AgR_14088	AgR_03583	5.7	17.0	0.0	-	30571714	32154688
	$F_{3:4}$/Tx714 early planted	7	AgR_12911	AgR_07177	6.7	11.3	0.0	-	135573519	138888510
	$F_{3:4}$/Tx714 early planted	10	AgR_07114	AgR_10075	5.8	5.7	0.0	-	140268347	141297647
Days to silk (days)										
	$F_{3:4}$ inbred	2	AgR_04201	AgR_14654	5.1	20.7	0.8	-0.3	63,819,796	68,700,035
	$F_{3:4}$ inbred	3	AgR_14903	AgR_11281	5.2	21.2	-0.9	0.5	5,589,411	5,853,098
Flag leaf height (cm)										
	$F_{3:4}$/Tx714 early planted	8	AgR_13166	AgR_00840	5.9	21.8	-2.1	-	119,721,769	-
	$F_{3:4}$/Tx714 late planted	10	AgR_13739	AgR_10061	5.7	29.5	-0.4	-	131,043,066	132,512,811
Ear height (cm)										
	$F_{3:4}$ inbred	8	AgR_16586	AgR_09612	6.2	27.5	-2.7	0.8	113,658,795	117,761,504
Grain moisture (%)										
	$F_{3:4}$/Tx714 early planted	10	AgR_01351	AgR_09927	6.2	22.9	0.3	-	-	13,787,744

LOD: log of odds ratio for QTL; PVE(%): the phenotypic variation explained by individual QTL; Add: the additive effect value; Dom: the dominance effect value.
Chr: chromosome; LeftPos and RightPos: the physical position of left and right markers according to Maize B73_RefGen_v2.
"-":non-available data

Table A5. QTLs mapped in LH82/LAMA-YC population using best linear unbiased predictions.

Trait	QTL	Chromosome	Position	LeftMarker	RightMarker	LOD	PVE(%)	Add	LeftPos (bp)	RightPos (bp)
Days to silk (days)										
	qDS7	7.0	42.0	QTV2	AgR_03110	21.4	6.8	0.0	164,955,163	164,987,683

Chr.:chromosome; LeftPos and RightPos: the physical position of left and right markers according to Maize B73_RefGen_v2.
LOD: log of odds ratio for QTL; PVE(%): the phenotypic variation explained by individual QTL; Add.: the additive effect value.
na: non-available. TC-Dry: testcross hybrid non-irrigation test; TC-Irg: testcross hybrid irrigation test.

Table A6. Consistent QTLs in Ki3/NC356 population and the candidate genes.

Trait	QTL	Chr.	Interval (Mb)
Days to anthesis (days)			
	qDA2	2	17.48-17.90
	qDA5	5	154.81-158.54
Days to silk (days)			
	qDS2	2	17.48-17.90
	qDS5	5	154.81-158.54
	qDS10	10	121.29-121.73
Plant height (cm)			
	qPH2	2	37.13-37.45
	qPH8	8	#-17.38
Flag leaf height (cm)			
	qFH2-1	2	#-12.07
	qFH2-2	2	19.99-20.21
	qFH3	3	12.13-13.27
Grain moisture			
	qGM8	8	164.04-164.81
Grain testweight			
	qTW3	3	6.43-6.94

#: unknown information; Chr. :Chromosome.

Table A7. Consistent QTLs in Tx740/NC356 population and the candidate genes.

Trait	QTL	Chr.	Interval (Mb)
	qDA9-2	9	136.79-137.79
	qDS2	2	63.82-68.70
	qDS3	3	5.59-5.85
	qFH10	10	131.04-132.51
	qEH8	8	113.66-117.76

Chr. :Chromosome.

R Code for the Ki3/NC356 population

```
library(qtl)

#loading input

Ki3NC356<-read.cross("csvr", "",estimate.map=FALSE)

summary(Ki3NC356)

#plot missing value

plotMissing(Ki3NC356)

#drop the individuals have >10% missing genotyping value

Ki3NC356<-subset(Ki3NC356, ind=(ntyped(Ki3NC356)>5338))

summary(Ki3NC356)

par(mfrow=c(1,2), las=1, cex=0.8)

plot(ntyped(Ki3NC356),ylab="No.typed Markers", main="No.types by individual")
```

```
#plotMissing(Ki3NC356)

#drop the difficult-to-call markers

nt.bymar<-ntyped(Ki3NC356,"mar")

todrop<-names(nt.bymar[nt.bymar<190])

Ki3NC356<-drop.markers(Ki3NC356,todrop)

plot(ntyped(Ki3NC356,"mar"), ylab="No.typed individuals", main="No.genotypes by
marker")

summary(Ki3NC356)

plotMissing(Ki3NC356)

#look for markers with distorted segregation patterns

gt<-geno.table(Ki3NC356)

gt[gt$P.value<0.05/totmar(Ki3NC356),]

#todrop<- rownames(gt[gt$P.value<1e-10,])

#Ki3NC356<-drop.markers(Ki3NC356,todrop)

#study individuals' genotype frequencies

g<-pull.geno(Ki3NC356)

gfreq<-apply(g,1,function(a) table(factor(a, levels=1:3)))

gfreq<- t(t(gfreq)/ colSums(gfreq))
```

```
par(mfrow=c(1,1), las=1)

for(i in 1:3)

plot(gfreq[i,], ylab="Genotype frequency", main=c("AA","AB","BB")[i],ylim=c(0,1))

##study pairwise marker linkages; look for switched alleles

Ki3NC356<-est.rf(Ki3NC356)

lg<-formLinkageGroups(Ki3NC356, max.rf=0.25, min.lod=3)

table(lg[,2])

checkAlleles(Ki3NC356, threshold=5)

rf<-pull.rf(Ki3NC356, what="lod")

plot(as.numeric(rf), as.numeric(lod), xlab="Recombination fraction", ylab="LOD
score")

#form linkagegroups use est.rf

Ki3NC356<-est.rf(Ki3NC356)

Ki3NC356<- formLinkageGroups(Ki3NC356, max.rf=0.25,
min.lod=3,reorgMarkers=TRUE,verbose=TRUE)

summary(Ki3NC356)

par(mfrow=c(1,1), las=1)

plotRF(Ki3NC356, alternate.chrid=TRUE,mark.diagonal=TRUE)

#form linkage group using a general likelihood ratio test
```

```
#Ki3NC356<- formLinkageGroups(Ki3NC356, max.rf=Inf,
min.lod=40,reorgMarkers=TRUE,verbose=TRUE)

#summary(Ki3NC356)

#par(mfrow=c(1,1), las=1)

#plotRF(Ki3NC356, alternate.chrid=TRUE,mark.diagonal=TRUE)

###orderMarkers across the genome

Ki3NC356<-orderMarkers(Ki3NC356,chr=1,window=4, use.ripple=TRUE,
map.function="kosambi", maxit=1000, sex.sp=F, verbose=T)

pull.map(Ki3NC356, chr=1)

summaryMap(Ki3NC356)

Ki3NC356<-orderMarkers(Ki3NC356,chr=2,window=4, use.ripple=TRUE,
map.function="kosambi", maxit=1000, sex.sp=F, verbose=T)

pull.map(Ki3NC356, chr=2)

summaryMap(Ki3NC356)

Ki3NC356<-orderMarkers(Ki3NC356,chr=3,window=4, use.ripple=TRUE,
map.function="kosambi", maxit=1000, sex.sp=F, verbose=T)

pull.map(Ki3NC356, chr=3)

summaryMap(Ki3NC356)

Ki3NC356<-orderMarkers(Ki3NC356,chr=4,window=4, use.ripple=TRUE,
map.function="kosambi", maxit=1000, sex.sp=F, verbose=T)
```

```
pull.map(Ki3NC356, chr=4)

summaryMap(Ki3NC356)

Ki3NC356<-orderMarkers(Ki3NC356,chr=5,window=4, use.ripple=TRUE,
map.function="kosambi", maxit=1000, sex.sp=F, verbose=T)

pull.map(Ki3NC356, chr=5)

summaryMap(Ki3NC356)

Ki3NC356<-orderMarkers(Ki3NC356,chr=6,window=4, use.ripple=TRUE,
map.function="kosambi", maxit=1000, sex.sp=F, verbose=T)

pull.map(Ki3NC356, chr=6)

summaryMap(Ki3NC356)

Ki3NC356<-orderMarkers(Ki3NC356,chr=7,window=4, use.ripple=TRUE,
map.function="kosambi", maxit=1000, sex.sp=F, verbose=T)

pull.map(Ki3NC356, chr=7)

summaryMap(Ki3NC356)

Ki3NC356<-orderMarkers(Ki3NC356,chr=8,window=4, use.ripple=TRUE,
map.function="kosambi", maxit=1000, sex.sp=F, verbose=T)

pull.map(Ki3NC356, chr=8)

summaryMap(Ki3NC356)

Ki3NC356<-orderMarkers(Ki3NC356,chr=9,window=4, use.ripple=TRUE,
map.function="kosambi", maxit=1000, sex.sp=F, verbose=T)
```

```
pull.map(Ki3NC356, chr=9)

summaryMap(Ki3NC356)

Ki3NC356<-orderMarkers(Ki3NC356,chr=10,window=4, use.ripple=TRUE,
map.function="kosambi", maxit=1000, sex.sp=F, verbose=T)

pull.map(Ki3NC356, chr=10)

summaryMap(Ki3NC356)

plotMap(Ki3NC356)

plotRF(Ki3NC356, alternate.chrid=TRUE, mark.diagonal = TRUE)

#SAVE FILE

Ki3NC356map<-pull.map(Ki3NC356)

tab<-map2table(Ki3NC356map)

setwd("")

write.csv(tab, "Ki3NC356map.csv")
```