MARKER ASSISTED SELECTION AND BREEDING FOR DESIRABLE THINNER PERICARP THICKNESS AND EAR TRAITS IN FRESH MARKET WAXY CORN GERMPLASM

BY

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DISSERTATION

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ABSTRACT

Kernel pericarp thickness and ear architectural traits are important selection criteria in fresh waxy corn breeding programs as they are associated with consumer sensory and visual preferences. An $F_{2:3}$ mapping population from the cross between South Korean inbreds BH20 and BH30 was developed in order to estimate genetic relationships among pericarp thickness traits and ear architectural traits, and to identify QTL regions for these traits through univariate and multivariate approaches. High correlations among pericarp thickness traits were detected and QTL regions associated with multiple pericarp thickness traits were identified. Through incorporating principal component analysis (PCA) of pericarp thickness traits and ear traits with QTL analysis, we detected PC-QTL regions that appear to have pleiotropic effects on multiple traits, particularly the pericarp traits on different parts of the kernel. The pericarp thickness QTL information was used to perform marker assisted selection to pyramid favorable QTL, as well as validate pericarp QTL. The MAS population was designed to try and maintain favorable ear traits by making crosses between lines chosen for favorable ear and pericarp thickness phenotypes and lines chosen for favorable QTL alleles for pericarp thickness traits. A few ear traits showed weak but favorable associations with pericarp thickness traits. Evaluation of the MAS population revealed that most selected QTL markers were significant for at least one pericarp thickness trait. Comparing groups of lines in the MAS population sorted by: phenotypes for thinner pericarp; favorable QTL alleles for pericarp thickness; and unfavorable alleles for pericarp thickness from MAS population, we found that in some cases that marker based selection might be effective for reducing pericarp thickness. Pyramiding significant favorable marker alleles showed reduction of pericarp thickness on all kernel regions. Since testcross performance (TP) is ultimately more important than per se line performance (LP), a testcross population was

generated for groups of selected lines from MAS population. This was done to enable assessment of the effect of groups of lines and different testers, and to compare LP with TP. Group1 with most favorable alleles showed significantly thinner pericarp than group 2 with fewest favorable alleles in testcross evaluation, regardless of tester. The TP with tester BH1030, which was the thinner pericarp testcross hybrid showed thinner pericarp than TP with tester BH1020. We found evidences that suggested the tester had dominance effects on reducing pericarp thickness in testcross population. In summary, pericarp thickness QTL information was useful for marker assisted selection of favorable loci within Korean germplasm, and therefore offers the potential to be useful for introgression of these favorable loci into more adapted U.S. germplasm. Weak but favorable relationships among pericarp thickness and some ear traits could be used collectively to improve overall features through independent selection in a fresh waxy corn breeding program.

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INTRODUCTION AND OVERVIEW

Waxy corn is commonly eaten fresh in Asian countries such as in China, Japan, Korea, and Philippines. Consumption of fresh waxy corn is somewhat similar to that for sweet corn, at about 25 days after pollination (DAP). The preference of East Asians for waxy-type rice such as *japonica* varieties contributes to their enjoying waxy corn for the tenderness and stickiness (Kim *et al*., 1994). Due to increasing demand and consumption of fresh waxy corn, the production of corn for livestock feed in South Korea decreased from 22,000 ha in 1986 to 20,000 ha in 2003. In contrast, the production of waxy corn for fresh eating increased considerably from 2,000 ha in 1986 to 15,000 ha in 2003 (GARES, 2005).

Due to the distinctive nature of the desired taste and sensory traits of waxy corn, which has a much more starchy and sticky texture than sweet corn, waxy corn breeding in South Korea for fresh eating is primarily focused on tenderness (Kim *et al.,* 1994). Early studies showed that greater pericarp thickness is negatively associated with sensory perception of tenderness (Ito and Brewbaker, 1981; Tracy and Galinat, 1987). Therefore, selection for thinner pericarp is a priority for enhancing tenderness in fresh waxy corn breeding.

Since waxy corn is enjoyed among Asians for fresh consumption, there is a potential fresh waxy corn market in the U.S. due to the increasing Asian-American population, particularly in Los Angeles and Chicago. Presently only a few farmers in California, New Jersey, and Illinois grow waxy corn for fresh eating. Therefore, developing an adapted, high yielding hybrid with taste quality characteristics that meets the U.S. market needs for human fresh consumption would provide new opportunity for increasing waxy corn production.

There are a number of popular fresh waxy corn varieties grown in Asian countries. Although traditional landrace waxy corn varieties in South Korea have been bred for

favorable fresh consumption properties for decades, these varieties have problems with lodging, lower grain yields, and late maturity. However, due to the market needs in South Korea, breeding waxy corn is largely focused on taste quality (CRDA, 1995). The waxy corn hybrid Yeonnong1 is commercially produced for the Korean fresh market for its tenderness, due to thin pericarp. Yeonnong1 is a hybrid of the cross of BH10 and BH20 waxy corn inbreds, which were derived from South Korean landrace varieties (Lee *et al*., 1994). Developing adapted waxy corn hybrids for U.S. fresh market consumption and acceptance will likely involve introgressing these taste quality traits from Asian varieties, such as South Korean waxy corn hybrid Yeonnong1, into adapted U.S. genetic backgrounds. Molecular markers are powerful tools for mapping genes in maize (Dudley, 1993; Ahn and Tanksley, 1993). Due to their simplicity, abundance, and distribution throughout the genome, SSRs became a major marker system utilized in maize (Smith *et al*. 1997). Quantitative trait loci (QTL) mapping which detects alleles controlling desirable quantitative traits provides useful information to enhance maize breeding through marker assisted selection (MAS) (Stuber *et al*., 1999). Therefore, MAS could be used in facilitating transfer of favorable alleles for taste quality and consumer preference in Asian germplasm into elite U.S. germplasm, and also enhance early generation identification of plants with desirable alleles in subsequent selection programs.

Due to the necessity to breed new fresh waxy corn hybrids for U.S. market, genetic research on preference traits, specifically pericarp thickness associated with tenderness perception, and ear traits relevant to yield and consumer preference was conducted on a population derived from South Korean germplasm. Since there is limited genetic information on the pericarp and ear traits related to consumer preference of fresh waxy corn germplasm, estimating genetic relationships among the traits, identifying and validating QTL for the traits,

and evaluating the testcross performance of the traits would be useful in selection programs designed to improving these traits in fresh waxy corn breeding programs.

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CHAPTER ONE

GENETIC AND QTL ANALYSIS OF PERICARP THICKNESS AND EAR ARCHITECTURE TRAITS OF WAXY CORN

ASTRACT

Kernel pericarp thickness is relevant to consumer sensory preference for fresh market waxy corn. Ear size and architectural traits impact yield and consumer visual preference. Therefore, pericarp thickness and ear traits are important selection criteria for breeding fresh market waxy corn. This research was conducted to better understand genetic control of these traits in popular South Korean germplasm now grown in Illinois. Pericarp thickness on five kernel regions, and ten inflorescence architecture traits were measured on ears from 264 $F_{2:3}$ families from a cross between Korean inbreds BH20 and BH30. All five pericarp thickness traits showed high heritabilities and were highly correlated. Multivariate Principal Components Analysis (PCA) revealed that just one principal component (PC) explained most of the total phenotypic variation. Univariate and PCA approaches were used to detect QTL. A number of univariate QTL were detected and most were associated with more than one kernel pericarp region. Four out of seven PC-QTL were located in chromosome positions where three or more pericarp thickness univariate QTL were detected. Conversely, three PC-QTL were found in regions with just a single or two univariate QTL, indicating that these QTL regions may be more important for overall pericarp thickness than suggested by univariate analysis. The PCA, QTL, and PC-QTL results indicate that pericarp thickness on different kernel regions may be controlled by common genes with pleiotropic effects. Additive effects of QTL for thinner pericarp thickness came from both BH20 and BH30. For ear architecture traits, heritability varied from 0.38 to 0.72, and several traits were correlated. The PCA

reduced these traits into three independent PCs, and all substantial component traits for these PCs were also significantly correlated. A number of univariate QTL were clustered closely, and some PC-QTL were detected in these regions. Some PC-QTL were found in chromosome regions where univariate QTL were not detected, again suggesting that these regions may have larger overall effects on ear architecture than suggested by univariate analyses. Collectively, these QTL may be useful for marker assisted introgression into germplasm more adapted to the U.S.

INTRODUCTION

Fresh waxy corn is consumed in Asian countries, usually at 25 days after pollination (DAP). Several waxy corn hybrids are very popular in the South Korean market and are enjoyed because of their consumer appeal and high taste quality (Park et al., 1992; Lee and Choe, 1995; Cha and Moon, 2000). Most of these hybrids, however, are poorly adapted in the U.S., exhibiting susceptibility to disease, lodging and low yields. Demand for fresh market waxy corn is growing in the U.S. due to the increasing Asian-American population (Magazine Publishers of America). Several waxy corn hybrids, including two very popular South Korean hybrids, have been introduced in U.S. for the Asian-American market, particularly for the metropolitan areas of New York, Chicago, and Los Angeles. More information is needed on traits relevant to fresh waxy corn consumer preference and yield performance in order to better enable breeding in the U.S. because of agronomic problems and of generally limited information on fresh waxy corn properties.

A number of taste and consumer preference characteristics had been studied on sweet corn (Ito and Brewbaker, 1981; Tracy and Galinat, 1987; Azanza et al., 1996; Simonne et al., 1999). Based on these studies, logical traits for fresh waxy corn selection efforts are high

kernel tenderness and desirable conformation of ear inflorescence architecture traits comprised by number of kernel rows, row configuration, tipfill, kernel width and depth, ear shape, and ear size (Simonne et al., 1999; Hallauer, 2001). The detection of chromosome regions controlling pericarp thickness and ear architecture traits in popular Korean germplasm may facilitate introduction of favorable alleles into more elite, U.S. adapted backgrounds.

Thinness of pericarp, the outer layer of maize kernel, is a major selection target for improvement of tenderness in sweet corn (Ito and Brewbaker, 1981), popping expansion of popcorn (Mohamed et al. 1993), and drying rate of shelled corn (Stroshine et al. 1987). Therefore thinness of pericarp is also a major target for improvement of tenderness in fresh waxy corn. Based on the studies on sweet corn hybrid (Tracy and Galinat, 1987) and pericarp thickness of waxy corn hybrids measured in this study, the preferred range of pericarp thickness for consumer preference purpose would be approximately 35 μm to 60 μm. Pericarp is composed mainly of corn fiber, which is not fermentable in conventional ethanol production. Thus, understanding genetic relationships for pericarp thickness among different regions of the kernel and estimating genetic control of pericarp thickness may help selection programs for thinner pericarp designed to increase ethanol production efficiency and influence processing of ethanol byproducts (Dien et al., 2002; Rausch and Belyea, 2002).

Pericarp thickness varies greatly from tender sweet corns (35 μm) to thick Corn Belt dents (200 μm) (Brewbaker et al. 1996). An earlier study showed pericarp thickness of sweet corn hybrids for fresh consumption ranged from 50 μm to 148 μm (Tracy and Galinat, 1987). Three morphological changes were reported to contribute to variation in pericarp thickness: number of pericarp cell layers, differential thickening of cells on germinal and abgerminal surfaces, and thickening of individual pericarp cell walls (Ito and Brewbaker, 1991). Differences in pericarp thickness have been reported to be inherited quantitatively, with

partial dominance for thin pericarp in some crosses (Helm and Zuber 1972). Generation mean analyses involving segregants from crosses of thin-pericarped sweet corn inbreds and thick-pericarped dent corn inbreds, suggested that a relatively low to moderate number of genes control variability in pericarp thickness (Ito and Brewbaker, 1991). The estimates ranged from 1.4 to 5.9 genes. Narrow-sense heritability averaged 55.2%, and significant epistatic effects were detected. Environment appears to show very little effect on pericarp thickness (Helm and Zuber, 1969). Three QTL on chromosomes 1, 2 and 6 were associated with variation in pericarp thickness in a cross between dent parent Hi31 and tropical flint parent Ki14 (Wang and Brewbaker, 2001).

Ear architecture traits are associated with consumer appeal and also yield components. Altering them may impact yield of waxy corn. Consumers prefer big ear size and a large edible portion. Therefore, the ear architectural traits such as longer ear length, and greater ear weight, kernel depth and kernel weight influence consumer appeal. Waxy corn hybrids are grown at relatively low population densities in order to get large and appealing waxy corn ears. Production of large appealing ears from hybrids grown at higher population densities may be attainable by selection. Because of low grain yield heritability, indirect selection for higher yield via ear traits with high heritability has been suggested to give greater gain on yield than direct selection for yield (Robinson *et al*., 1951).

Few, if any, quantitative trait loci (QTL) studies have been performed on pericarp thickness and ear inflorescence architecture traits of fresh waxy corn varieties. Mapping QTL for pericarp thickness and ear traits followed by conventional and marker assisted selection (MAS) may be appropriate for a U.S. fresh waxy corn breeding program that wishes to introgress popular Korean germplasm. QTL analyses may also help our understanding of any genetic relationships among pericarp thickness and ear inflorescence architecture traits. In this study, phenotypic data on a set of five pericarp thickness traits and ten ear inflorescence

traits were collected. Since the pericarp traits along with some ear traits are highly correlated, multivariate analysis can be used by taking the correlation structure of data into account. Mapping QTL incorporating correlation structure of data may increase statistical power of detecting QTL and improve the precision of parameter estimation. This mapping method may also be useful to test a number of meaningful hypotheses involving multiple traits (Jiang and Zeng, 1995).

Principal component analysis (PCA) is a widely used multivariate method used to reduce the dimensionality of a data set with a large number of correlated variables, while losing little of the variation in the original data (Jolliffe, 1986; Smith, 2002; Upadyayula *et al*., 2006). PCA can be used to decompose the correlated variables into an uncorrelated smaller set of variables. Orthogonal linear combinations of traits, the principal components (PCs), are the eigenvectors of the eigenvalues of the phenotypic covariance matrix. The PCs themselves can be considered as traits, amenable to QTL detection (Jiang and Zeng, 1995; Upadyayula *et al*., 2006).

The objectives of the research were to (1) determine the heritabilities of and correlations among five pericarp thickness traits and ten ear architecture traits; (2) examine the genetic relationships among traits using PC analysis; (3) and detect QTL for pericarp thickness and for ear traits using both univariate and PC approaches.

MATERIALS AND METHODS

Genetic Material and Field Evaluation

The BH20 and BH30 are waxy corn inbreds derived from landrace varieties in South Korea, bred for their high-taste quality and good combining ability. A hybrid between BH20 and BH30 has been grown in South Korea and was introduced to the U.S. Asian-American

market for fresh consumption in 2005. The average pericarp thickness of this F_1 is about 53 μm.

The BH20 and BH30 inbreds were crossed and self pollinated to produce 264 $F_{2:3}$ families at the University of Illinois Research and Education Center in Urbana Illinois in 2004. The 264 $F_{2:3}$ families were grown at Illinois Crop Improvement winter nursery in Puerto Rico (2004-2005) where ten plants per row were sib-mated to create F_3 sib-mated families. The purpose of the sib-mating was to increase seed quantity for replicated trials without additional inbreeding.

Seed of the bulked sib-mated 264 F_3 families from Puerto Rico were planted in 2005 and 2006 at the University of Illinois Research and Education Center in Urbana Illinois in a two-replicate 44x6 alpha (0, 1) design. Each family was over-planted by being, grown in single-row plots 15 feet in length and then thinned to 15 plants per plot to provide equal competition. Five to seven self pollinated ears from within each plot were sampled to measure phenotypic traits.

Phenotypic Data Collection

Pericarp thickness was measured on five different regions of the kernel (Table 1.1). Pericarp thickness of the upper and lower portions of germinal and abgerminal regions were measured using method of Helm and Zuber (1972) while crown region was measured using method of Wolf et al. (1969), as modified by Martin, Loesch, and Wiser (1980) (Figure 1.1). The thickness was measured with a Mitutoyo digital micrometer on five randomly chosen kernels per sample.

Pericarp thickness data were collected from several different generations and sources: BH20, BH30, and F_1 seeds of the cross between BH20 and BH30 produced in 2003; $F_{2:3}$ seeds from F_2 plants self-pollinated in Illinois in 2004; sib-mated seeds from $F_{2:3}$ families

grown in Puerto Rico in 2005; and self-pollinated seeds from two replicates of sib-mated F_3 families in 2005 and in 2006.

Ear architecture traits measured were the length of the ear (CL), the number of kernels per row (NK), number of rows per ear at the middle of the ear (NR), the length of a sample of ten contiguous kernels in the middle of the ear (KT), the diameter of ear before shelling (ED) and the diameter of the cob after shelling (CD). The diameters were measured on the middle of the ear and cob using a caliper. Kernel depth (KD) was calculated by subtracting the cob diameter from the ear diameter. Ear weight (EW), cob weight (CW), and weight of 100 kernels (KW) were also recorded (Table 1). The ear traits were measured on five randomly selected ears per family. Ear trait data were collected from different generations: ears from F_2 plants self-pollinated in Illinois in 2004; sib-mated ears from $F_{2:3}$ families grown in Puerto Rico in 2005; and self-pollinated ears from two replicates of sib-mated F_3 families in 2005 and in 2006.

Phenotypic Data Analysis

The adjusted plot means for each year keeping the replication separate were calculated on phenotypic data measured on pericarp thickness and ear traits. Analysis was performed using SAS version 9.1 (SAS Institute Inc., Cary, NC) based on the model: $y_{ijklm} = \mu + \alpha_i + \beta_{j(i)} + \delta_{k(ij)} + \gamma_l + (\alpha\gamma)_{il} + \varepsilon_{ijkl}, + \varphi_{(ijl)m}$, where y_{ijklm} represents the value of individual kernels or ears within a plot, α_i the effect of *i*th environment, $\beta_{j(i)}$ the effect of *j*th replication in the *i*th year, $\delta_{k(ij)}$ the effect of *k*th block in *j*th replication of *i*th year, γ_l the effect of the *l*th family, $(\alpha\gamma)_{il}$ the effect of *i*th year by *l*th family interaction, and ε_{ijkl} represents residual error. Since values were taken from five random kernels per family per replication for pericarp thickness traits and five random ears per family per replication for ear traits except the data from 2004, kernels and plants were also included in the model as subsample, $\varphi_{(ijl)m}$ to get

more precise estimate of means. The PROC MIXED command was used by setting all the effect random and subsequent analyses were done on least square plot means.

The adjusted means from each year were pooled together to get a grand adjusted mean across years. Combined data was used for the QTL analyses consisting of the generations from four environments: data from $F_{2:3}$ ears grown in Illinois in 2004; data from sib-mated F_3 families grown in Puerto Rico in 2005; and data from two replicates of self pollinated F_3 sib-mated families in 2005 and in 2006 in Illinois. The adjusted data was calculated using the PROC MIXED procedure using the DDRM=KR option to adjust unbalanced data. Heritabilities and coefficients of correlation for the traits were calculated based on the adjusted means. Broad-sense heritability ($\hat{h}^2_B$) was estimated by 90% confidence intervals according to Knapp (1986).

Principal component analysis (PCA) was performed on correlation, rather than covariance matrices to eliminate measurement scale differences among traits. Eigenvalues (λ), accounting for the proportion of the variation attributable to each PC were calculated. Principal components (PCs) with the largest λ were then identified as those that accounted for the most of the total variation of the data. The PC loadings were examined to identify phenotypic variables with a substantial association with each selected PC. Loadings with absolute values > 0.3 were judged substantial. The PC scores were then calculated for each family (Upadyayula *et al*., 2006). The score data were then used for PC-QTL mapping.

QTL Analysis

A linkage map was constructed using 100 polymorphic Simple Sequence Repeats (SSR) markers on 264 $F_{2:3}$ families derived from BH20 and BH30. The map comprised a total genome length of 1281 centimorgans (cM) and an average distance between markers of 13 cM.

The QTL analyses were performed on a combined data set from four environments measured for pericarp and ear traits. QTL were positioned, and their effects were estimated by composite interval mapping (CIM) (Zeng, 1994; Jansen et al., 1994) by PLAB QTL version 1.2 (Utz and Melchinger, 1996) on both individual traits and PC scores. The data were not transformed for QTL analysis since the transformation of data may lead to reduced power of detection of real gene effects (Mutschler *et al*., 1996; Jampatong *et al*., 2002). Cofactors for CIM procedure were selected using the COV SEL command in PLAB QTL. The final model was selected by stepwise regression that minimized Akaike's information criterion with penalty = 3.0. The genome-wide threshold LOD value to determine significance of a QTL effect was based on the likelihood ratio value obtained by 1000 permutations of the data (Churchill and Doerge, 1994). A genome-wise significance cut-off level was set at 0.30 as discussed from previous investigations (Upadyayula *et al*., 2006). Only those QTL that were significant in the final multiple regression model were reported.

The phenotypic variation accounted for by individual QTL was estimated by the square of the partial correlation coefficient (R^2) from a final multiple regression model. This value was calculated as the coefficient of determination of a specified QTL conditional on all other significant QTL (Utz and Melchinger, 1996). The phenotypic variation ($\hat{\sigma}_p^2$) accounted for by all QTL (adjusted R^2) was estimated in accordance with Hospital *et al*. (1997). The genotypic variation ($\hat{\sigma}_g^2$) explained by the model (adjusted P) was estimated by dividing the adjusted R^2 by heritability (Dudley, 1993).

Since a number of traits were highly correlated, we carefully examined closely linked QTL. If more than one peak of a univariate QTL interval was present in an approximately 20cM interval, the postions of these peaks were collapsed into what we termed as common QTL intervals. The QTL results for PC data were compared with univariate QTL intervals

within the same 20cM interval to identify potentially common QTL (Visscher et al., 1996; Groh et al., 1998; Melchinger et al., 1998).

RESULTS

Phenotypic Data Analysis

Pericarp thicknesses of the upper germinal, lower germinal, upper abgerminal, lower abgerminal, and crown regions on BH30 kernels were significantly thinner than the corresponding regions of BH20 kernels (Table 1.2). Pericarp thicknesses of all five regions of kernels on BH20 and on BH30 were significantly different from one another, except for the upper abgerminal versus crown regions of BH30 kernels. The germinal region of BH20 kernels was thinner than the abgerminal region. In contrast, the BH30 germinal region was thicker than abgerminal region. Average pericarp thickness of the five regions of the mapping population across the years was 54.1μm. This value was similar to the midparental value of 53.7μm. The average difference between the upper and lower sections of the mapping population was significant, but no significant difference between germinal and abgerminal averages was observed. The pericarp thickness of crown region was significantly thinner than all of the other regions (Table 1.3).

The analyses of variance results revealed significant ($P<0.01$) differences among families for all traits. Environment effects were highly significant ($P<0.01$) for pericarp thickness and ear traits except for number of rows per ear (NR). The family x environment interaction was significant for all traits.

Heritability and Correlation Estimates

Heritability ($\hat{h}^2_B$) estimated for pericarp thickness was relatively high ranging from 0.70 for crown (CWN) to 0.81 for lower germinal (LG) (Table 1.3). Phenotypic correlations among the five pericarp thickness regions were positive and highly significant (Table 1.4).

Ear trait heritabilities ranged from 0.38 for kernel depth (KD) to 0.72 for cob weight (CW) (Table 1.3). Cob length (CL) was positively correlated with all the ear traits except KD. Ear diameter (ED) was positively associated with all the ear traits. Cob diameter (CD) showed significant positive correlation with all the ear traits except KD. The KD trait was positively associated with ED, number of rows/ear (NR), ear weight (EW) and kernel weight (KW). Number of kernels/row (NK) exhibited positive correlation with all ear traits except KD, kernel thickness (KT) and KW. The KW was significantly and positively correlated with all of the ear traits except NK but significantly and associated with NR negatively. The NR showed significant associations with all of the traits except KT. The KT showed positive correlation with CL, ED, CD, CW and KW. The EW was positively associated with all of the ear traits except KT. The CW was also positively correlated with all of the traits except KD. All five pericarp thickness traits showed positive significant correlation with both CD and CW (Table 1.4).

Principal Component Analysis

The first PC (PC1) for pericarp thickness accounted for 88.5% of the total variation. Loadings for the pericarp traits were remarkably similar and ranged from 0.44 to 0.46 (Table 1.5). For ear traits, three PC with $\lambda>1$ were selected. These three PC explained 73.7% of the total phenotypic variation. The PC1 explained 40.0% of the total phenotypic variation with CL, NK, ED, CD, EW, and CW having substantial positive loading values. The PC2 explained 17.6% of total variation with loadings for KT and KW being positive-substantial

and those for CL and NK negative-substantial. The PC3 explained 16.1% of total variation with positive-substantial loading for CL and KW and with negative-substantial loadings for ED, CD and NR (Table 1.5).

Univariate QTL Analysis on Pericarp Thickness Traits

In total, thirty-three individual QTL were detected for the five pericarp measurements. The number of QTL for pericarp thickness ranged from 5 to 9 for the different kernel regions. The upper germinal region showed the highest $\hat{\sigma}_g^2$, 45.6%, in the final QTL models. Most of the significant additive effects for thin pericarp came from BH30, the thinner of the two parents. However, for several QTL, the BH20 allele was associated with thin pericarp (Table 1.6).

Ten common QTL intervals were associated with variation in pericarp thickness for two or more kernel regions. Common QTL intervals significant for all five kernel regions were detected on chromosome 2 and 3 in the 98-112cM and 14-34cM intervals, respectively. Chromosome 3, 80cM (same peak for LG, and LA) was associated with two kernel regions. A common QTL interval on chromosome 9, 76-82cM, was associated with pericarp thickness variation in three kernel regions. Three common QTL intervals on chromosome 4, 26-34cM, 76cM (same peak for UA, and CWN), and 98-100cM were associated with pericarp thickness variation in two kernel regions. The common QTL interval on chromosome 8 at 6-8cM was also associated with two kernel regions. All of the thin additive effects of these six common QTL intervals were associated with BH30 alleles. The common QTL interval at chromosome 1, 106-108cM, was associated with three regions. Chromosome 10, 130cM (same peak for UC, and LG) was associated with two kernel regions (Table 6 and Table 10).

Univariate QTL Analysis on Ear Traits

Fifty-one QTL were detected across all ear traits evaluated on the 264 F_3 families derived from BH20xBH30. The final QTL model for KW contained nine QTL. This model was the highest for $\hat{\sigma}_g^2$ (56.2%). The most positive additive QTL effects were associated with the BH20 alleles. The QTL on chromosome 10 at 62cM exhibited the largest $\hat{\sigma}_p^2$ (12.9%) with positive effect from BH20. Eight QTL for CW were detected explaining $\hat{\sigma}_g^2$ of 37.1%. The QTL on chromosome 4 (34cM) exhibited the largest $\hat{\sigma}_p^2$. Additive effects for high CW were attributable to both parents. Only two QTL ($\hat{\sigma}_g^2$ = 20.2%) were found for EW on chromosome 2 (78cM) and chromosome 7 (68cM), and both had additive effects associated the BH30 alleles (Table 1.7).

For KT, six QTL were found ($\hat{\sigma}_g^2$ = 45.4%). The QTL on chromosome 6 at 124cM exhibited the largest $\hat{\sigma}_p^2$ (6.5%). Most QTL alleles for large KT were associated with BH20, except QTL on chromosome 9 (0cM). For NR, five QTL were detected with $\hat{\sigma}_g^2$ =40.0%. Four out of five QTL additive effects for larger number of rows were associated with BH30 allele. Four QTL were detected for NK with $\hat{\sigma}_g^2$ = 29.1%. Three QTL alleles for high NK were associated with BH30. Three QTL were detected for CL with $\hat{\sigma}_g^2$ = 19.2%. Five QTL for ED and seven QTL for CD were found with $\hat{\sigma}_g^2$ = 30.9% and 36.1% respectively. Additive effects for increasing ED and CD were contributed from both BH20 and BH30. Only two QTL were found for KD with $\hat{\sigma}_g^2$ = 14.8% (Table 1.7).

Fourteen common QTL intervals were associated with two or more ear traits. The common QTL interval with QTL associated with the highest number of ear traits were found on chromosome 3 72-90cM for ED, KD, KT, NR, and CW, and on chromosome 4 34-52cM for CL, CD, NK, NR, and CW. The common QTL interval on chromosome 3 (72-90cM)

included four QTL with additive effects for larger values associated with BH20 allele. Only NR effects for higher row number were associated with BH30 allele. On chromosome 4 the common QTL interval additive effects were all associated with BH20. The common QTL interval chromosome 10 (144-148cM) was associated with 3 ear traits. Positive additive effects for CL, ED, and NK were all associated with the BH30 allele. Four common QTL intervals associated with three traits were found on chromosome 2 72-78cM, chromosome 6 124-134cM, chromosome 7 66-78cM and chromosome 8 2-10cM. The common interval on chromosome 2 had QTL for ED, EW, and KW with additive effects for larger value associated with BH30. The common interval on chromosome 6 had QTL for CD, KT and KW with additive effect for larger values associated with BH20. The common QTL interval on chromosome 7 had QTL for CD, EW, and CW with positive additive effects associated with BH30. The common interval on chromosome 8 had QTL for NK, KT, and CW with additive effects for larger value associated with both parents. Seven common intervals were associated with two traits. Some intervals such as chromosome 2 0cM for NK and KW, chromosome 5 0cM for CW and KW, chromosome 5 110cM for CD and NR, and chromosome 10 62cM for KT and KW consisted of single peaks (Table 10).

PC-QTL Analysis

Seven PC1-QTL were detected with $\hat{\sigma}_p^2$ = 29.8%. The PC-QTL with greatest $\hat{\sigma}_p^2$ (10.6%) was on chromosome 2 at 104cM. Most additive effects for thin pericarp were associated with BH30, except for two PC-QTL on chromosome 1 and 6 (Table 1.8). Ear trait PC1 was associated with CL, ED, CD, NK, EW and CW. Nine QTL were detected with $\hat{\sigma}_p^2$ = 32.7%. The PC-QTL on chromosome 4 at 36cM had the highest $\hat{\sigma}_p^2$ (13.6%) with additive effects from BH30. For PC2, seven QTL were detected with $\hat{\sigma}_p^2$ = 36.6%. Loadings on PC2

contrast KT and KW with CL and NK. The PC-QTL on chromosome 8 at 12cM exhibited the largest $\hat{\sigma}_p^2$ (12.1%). Most of the PC2-QTL additive effects were associated with BH20, except for the PC-QTL on chromosome 4. Eight PC3-QTL were detected with $\hat{\sigma}_p^2$ = 33.3%. Ear trait PC3 contrasts CL and KW with ED, CD and NR. The PC-QTL on chromosome 5 at 104cM exhibited the highest $\hat{\sigma}_p^2$. Most of the additive effects were associated with BH20 except for the PC-QTL on chromosome 3 (Table 1.9).

DISCUSSION

Correlations among the five different pericarp thickness traits were positive and highly significant. PCA results indicated that most of total $\hat{\sigma}_p^2$ (88.5%) was explained by one PC and that the five pericarp trait loadings were all positive and nearly equal. These results suggest that the pericarp thickness variations on different regions of a kernel may be controlled by common genes with pleiotropic effects. If so, pericarp thickness on different regions can essentially be considered a single trait. Selection for thinner pericarp thickness on any part of the kernel will likely results in thinner pericarp thickness for all regions. A number of QTL for three or more pericarp regions mapped in very close proximity. Other QTL, however, for only one or two pericarp regions were mapped in close proximity. If pericarp thickness is in fact a single trait, this would suggest that we increased the number of QTL detected overall by assessing all five regions of kernel.

The high correlations among several ear traits provided useful information relevant to improving consumer preference and yield component traits via direct and indirect selection in lower population densities. Among the correlations, it was notable that the correlation between cob diameter and kernel depth was not significant. Since kernel depth was calculated

from ear diameter and cob diameter, we expected a correlation between kernel depth and cob diameter. Our result suggests that genetic control for kernel depth is somewhat independent of genetic control for cob diameter in this genetic background.

The ear trait PC1 explained 40.0% of total phenotypic variation and had substantial positive loadings for CL, ED, CD, NK, EW and CW (Table 1.5). These traits are positively correlated and associated with overall ear development. Large ear size and edible portion are related to increases in cob length, ear diameter, cob diameter, number of kernels per row, ear weight and cob weight. Thus selecting for PC1-QTL may be effective in fresh waxy corn breeding.

The PC2 for ear architecture explained 17.6% of total phenotypic variation and the traits with substantial loadings were CL, NK, KT and KW. The CL showed a positive correlation with NK. The KT showed a negative correlation with NK but was positively correlated with KW. Increasing KT therefore could increase the KW through increasing kernel size at the expense of NK. The PCA results also agreed with this interpretation where PC2 largely involved KT (loading 0.44), KW (loading 0.53), CL (loading -0.41), and NK (loading -0.48) (Table 1.5). These traits are associated with ear length development and indirectly impact kernel weight. The association of thicker kernel with fewer kernels per row is not surprising. However, thicker kernels were also associated with shorter cob length in this genetic background, indicating that a simple trade-off of KT and NK may be involved.

The ear trait PC3 explained 16.1% of total phenotypic variation, and the substantial loadings were KW (0.30), CL (0.35), ED (-0.40), CD (-0.32), and NR (-0.61) (Table 1.5). The KW was negatively correlated with NR, ED and CD was weakly but positively correlated with CL, agreeing with the PC3 result. These traits are reflective of ear and cob thickness development and also impact kernel weight. Decreasing NR would likely be associated with increasing KW and KT in this germplasm, resulting in preferable to consumers (Table 1.4).

The correlations suggest that multiple trait selection could produce a combination with high consumer appeal with a larger edible portion with smaller cob size while maintaining larger kernels and longer ear length.

More QTL for pericarp thickness traits with larger overall R^2 were identified in this study in comparison to a previous study that found QTL on chromosome 1, 2 and 6 that linked to umc132, umc198 and umc185 respectively. All QTL were located on the different positions in this study compared to the loci found from the previous study by Wang and Brewbaker (2001).

Most individual QTL alleles for thin pericarp came from BH30. However, several QTL alleles for thin pericarp came from BH20. Transgressive segregation for pericarp thickness was observed consistently with both parents contributing alleles that reduce pericarp thickness. Consequently it should be possible to increase tenderness in more adapted germplasm by introgressing favorable alleles from both parents. However, on chromosomes 6 and 8, closely linked QTL with opposite effects for pericarp thickness coming from each parent were evident. In this case it will be necessary to break repulsion phase linkages to pyramid the favorable alleles.

A number of QTL encompassing different traits for pericarp and ear traits were clustered within an approximately 20cM intervals. Pleiotropism may be the cause. Multivariate analysis for related traits may help with understanding genetic control of multiple traits (Jiang and Zeng, 1995). Reducing highly correlated variables into smaller sets of orthogonal principal components through PCA may increase power of detecting QTL by reducing genome-wide false positive detection rates. The PC-QTL analysis carried out for each PC provided similar results to QTL analysis of individual trait data in many cases while also detecting additional genomic regions that were not detected in individual analysis of ear traits. Using PCs rather than using a univariate trait approach, some QTL became more

significant with increases in R^2 and LOD. Detection of PC-QTL may increase chances of detecting QTL associated with multiple traits.

Six PC1-QTL for pericarp thickness mapped to intervals containing more than one significant univariate QTL. The PC1-QTL on chromosome 6, however, was mapped closely to only a single univariate QTL. For ear traits, twenty-four PC-QTL were detected on three PCs. Most PC-QTL corresponded to QTL intervals associated with two or more ear traits in the univariate QTL analysis, again supporting a hypothesis of multi-trait, pleiotropic QTL. Some PC-QTL corresponded to QTL from single univariate QTL, yet the results suggest that the power of detecting QTL associated with all correlated traits appears to be increased by PCA. Selection based on pleiotropic QTL would likely enhance the efficiency of MAS. Selection of fewer QTL with pleiotropic effects would reduce effort and complexity.

Multi-trait QTL were found in several regions. The interval in bin 7.02-7.03 (66-78cM) was associated with QTL involving three ear traits; CD, EW and CW. In the previous research, QTL in bin 7.02 were detected for number of kernels per row in the IHOxB73 and IBM populations (The Maize Inflorescence Project Data Portal, 2006). This interval includes the inflorescence architecture mutant *ramosa1* (*ra1*) in bin 7.02 associated with the ear branching (Vollbrecht and Sigmon, 2005), suggesting that the multi-trait QTL could be a manifestation of pleiotropic effects at the *ra1* locus.

The QTL interval in bin 3.03-3.04 at 72-90cM was associated with the five ear traits, ED, KD, KT, NR, and CW, coinciding with QTL found in the IHOxB73 population for kernel thickness and number of kernel rows per ear traits (Upadyayula et al., 2006). The PC3-QTL with substantial loadings for KW, CL, ED, CD, and NR also mapped to the 78cM position with high R^2. This QTL genomic region includes the inflorescence architecture mutants *liguleless3 (lg3)* and *tassel seed4 (ts4)* in bin 3.04, making the regions attractive for association mapping of candidate genes. The QTL region in 3.04 may be important for

breeding ear traits on consumer preference associated with kernel and cob size. The QTL interval in bin 4.03-4.05 (34-52cM) was associated with CL, CD, NK, NR and CW. The PC-QTL detected in bin 4.03 (36cM) for PC1 possessed substantial loadings for CL, ED, CD, NK, EW, and CW. This QTL interval is located in the same bin as *fasciated ear2* (*fea2*) locus which affects ear meristem growth (Taguchi-Shiobara et al., 2001; MaizeGDB).

Some PC-QTL for different ear trait PCs mapped closely together. All three PC-QTL mapped to 6.05-6.06 114-134cM, and three univariate QTL for CD, KT and KW also mapped to the 124-134cM region. These QTL may comprise a cluster of several genes individual traits or a single QTL with pleiotropic effects. The PC2-QTL, and PC3-QTL mapped closely to bin 8.04-8.05 (4-12cM) as did three univariate QTL for NK, KT and CW. The number of kernels per row trait QTL was found in this region in the IBM and (IHOxB73) mapping populations (The Maize Inflorescence Project Data Portal, 2006)

Many QTL were found in this study for pericarp thickness traits responsible for tenderness and ear traits responsible for consumer preference. We found several desirable QTL in bins 1.10, 2.06, 3.00, 4.01, 4.07, 6.05, and 9.03 for thinner pericarp thickness with favorable alleles from both parents. Conversely, we also found desirable QTL regions for increasing ear traits in bins 1.08, 2.05-06, 3.03-04, 4.05, 6.06, and 7.02-03 with favorable alleles from both parents. These QTL results should aid in the introgression of favorable traits from Korean lines into adapted U.S. germplasm and stream line conventional and MAS selection efforts.

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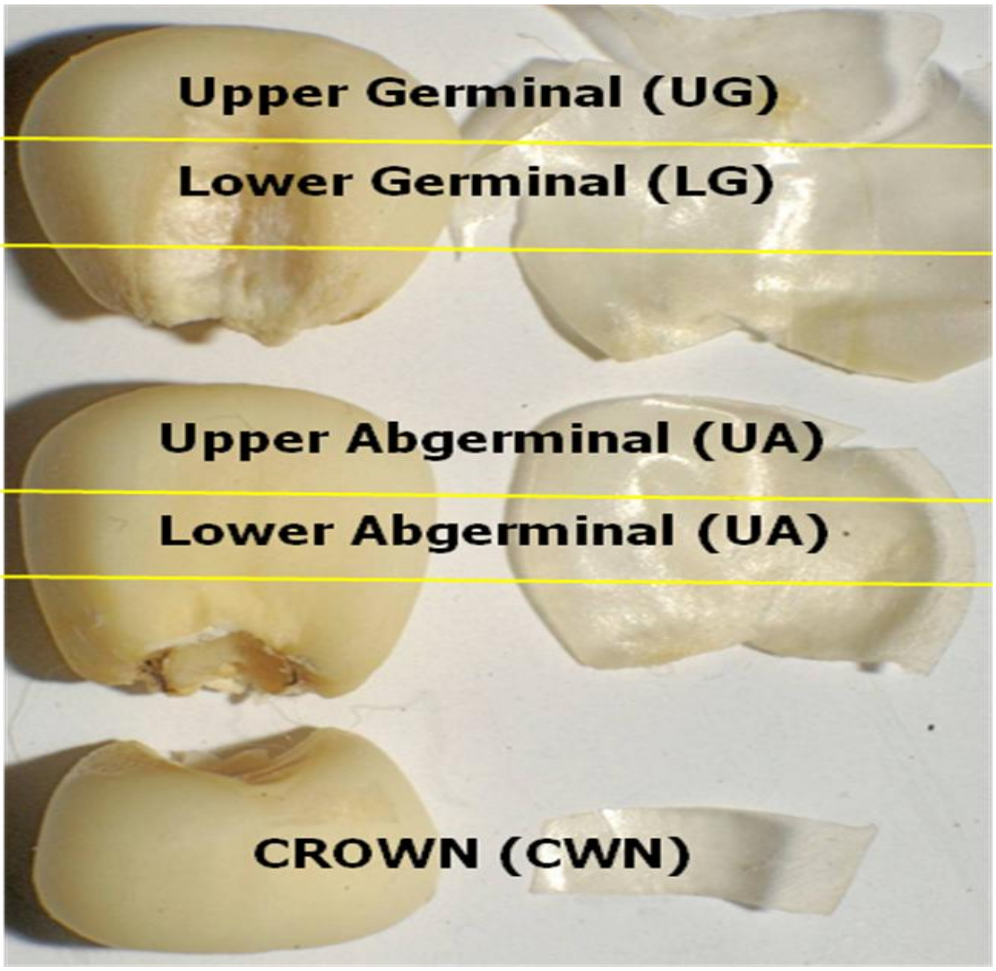
TABLES AND FIGURES

Figure 1.1 Five regions of pericarp thickness traits.



Germinal is the side with the germ and abgeminal is the opposite side of the germinal.

Table 1.1 List of pericarp thickness and ear inflorescence architecture trait measurements.

Trait	Abbreviation	How measured/calculated
Pericarp thickness traits		
Upper germinal	UG	Thickness of upper germinal part of pericarp in µm
Lower germinal	LG	Thickness of lower germinal part of pericarp in µm
Upper abgerminal	UA	Thickness of upper abgerminal part of pericarp in µm
Lower abgerminal	LA	Thickness of lower abgerminal part of pericarp in µm
Crown	CWN	Thickness of crown part of pericarp in µm
Ear achitecture traits		
Cob length	CL	Length of the cob in mm
Number of kernels/row	NK	Average number of kernels in two rows on opposite sides of cob
Number of rows/ear	NR	Number of rows per ear at the middle of the ear
Kernel thickness	KT	Length of ten kernels at the middle of the ear in cm
Ear diameter	ED	Diameter of the ear before shelled at the middle of the ear in mm
Cob diameter	CD	Diameter of the cob after shelled at the middle of the cob in mm
Kernel depth	KD	Ear diameter - Cob diameter
Ear weight	EW	Weight of the ear before shelled in g
Cob weight	CW	Weight of the cob after shelled in g
Kernel weight	KW	Weight of 100 kernels in g

Table 1.2 Means of parents BH20 and BH30, and $F_{1:2}$ derived from BH20xBH30 on pericarp thickness traits.

Trait	BH20	BH30	F_1
Upper germinal (UG) (µm)	58.63±2.90[§] a[†]	45.91±0.55 a	57.00±2.28 a
Lower germinal (LG) (µm)	73.00±2.17 b	53.27±1.13 b	64.61±2.76 b
Upper abgerminal (UA) (µm)	64.55±1.64 c	34.00±1.28 c	49.11±2.49 c
Lower abgerminal (LA) (µm)	91.55±0.62 d	38.09±1.47 d	62.17±3.37 ab
Crown (CWN) (µm)	46.73±1.70 e	31.36±0.72 c	35.78±1.82 d
Average (µm)	66.89±1.59 f	40.53±0.89 d	53.73±2.44 e

§ Standard errors are attached.

† Means within a column followed by the same letter are not different at the 0.05 probability level.

Table 1.3 Mean, range and heritability estimates for pericarp thickness traits of 264 F_3 families derived from BH20xBH30 measured in four environments from 2004-2006.

Trait	Mean	H^2_B
Upper germinal (UG) (µm)	50.73 ± 0.31§	0.74
Lower germinal (LG) (µm)	66.22 ± 0.35	0.82
Upper abgerminal (UA) (µm)	49.90 ± 0.30	0.80
Lower abgerminal (LA) (µm)	65.30 ± 0.42	0.78
Crown (CWN) (µm)	37.72 ± 0.23	0.70
Cob length (CL) (mm)	145.7 ± 0.06	0.64
Ear diameter (ED) (mm)	34.30 ± 0.10	0.66
Cob diameter (CD) (mm)	21.26 ± 0.05	0.63
Kernel depth (KD) (mm)	12.92 ± 0.11	0.38
Number of kernels per row (NK)	28.78 ± 0.12	0.56
Kernel thickness (KT) (cm)	4.87 ± 0.01	0.47
Number of rows per ear (NR)	11.16 ± 0.03	0.56
Ear weight (EW) (g)	69.78 ± 0.90	0.64
Cob weight (CW) (g)	9.02 ± 0.08	0.72
Kernel weight (KW) (g)	20.98 ± 0.11	0.70

§ Standard errors are attached.

Table 1.4 Phenotypic correlation coefficients among pericarp thickness and ear traits for 264 F_3 families derived from BH20xBH30 measured in four environments from 2004-2006.

	UG	LG	UA	LA	CWN	CL	ED	CD	KD	NK	KT	NR	EW	CW
LG	0.85**													
UA	0.90**	0.81**												
LA	0.80**	0.82**	0.89**											
CWN	0.92**	0.82**	0.92**	0.83**										
CL	0.13*	0.11	0.08	0.00	0.07									
ED	0.04	0.08	0.04	0.02	0.03	0.15*								
CD	0.20**	0.20**	0.18**	0.16*	0.20**	0.27**	0.65**							
KD	-0.12	-0.08	-0.11	-0.13*	-0.14*	-0.05	0.69**	-0.08						
NK	0.10	0.06	0.05	-0.06	0.07	0.85**	0.15*	0.25**	0.01					
KT	0.15 *	0.19**	0.12	0.18**	0.14*	0.16*	0.19**	0.36 **	-0.06	-0.02				
NR	0.09	0.12	0.06	0.01	0.02	0.17*	0.49**	0.45**	0.23**	0.19**	-0.08			
EW	-0.05	-0.11	-0.08	-0.11	-0.06	0.32**	0.51**	0.28**	0.40**	0.35**	0.09	0.18 **		
CW	0.23**	0.19**	0.19**	0.12*	0.20**	0.56**	0.42**	0.58**	0.04	0.49**	0.33**	0.24**	0.44**	
KW	-0.04	-0.05	-0.05	0.00	-0.05	0.13*	0.36**	0.21**	0.28**	0.05	0.43**	-0.19**	0.50**	0.31**

*, ** Phenotypic correlation was significant at the 0.05 and 0.01 probability level, respectively.
UG: Upper germinal, LG: Lower germinal, UA: Upper abgerminal, LA: Lower abgerminal, CWN: Crown.
CL: Cob length, ED: Ear diameter, CD: Cob diameter, KD: Kernel depth, NK: Number of kernels/row, KT: Kernel thickness,
NR: Number of rows/ear, EW: Ear weight, CW: Cob weight, KW: Kernel weight.

Table 1.5 Parameters associated with the first principal component on pericarp thickness and ear traits and their loadings.

Parameter	PC1
Eigenvalue (λ)	4.42
% of total variation	88.47
Pericarp thickness trait loadings	
Upper germinal (UG)	0.45[$]
Lower germinal (LG)	0.44
Upper abgerminal (UA)	0.46
Lower abgerminal (LA)	0.44
Crown (CWN)	0.45

Parameter	PC1	PC2	PC3
Eigenvalue (λ)	3.60	1.58	1.45
% of total variation	40.0	17.6	16.1
Ear trait loadings			
Cob length (CL)	**0.36**	**-0.41**	**0.35**
Ear diameter (ED)	**0.37**	0.21	**-0.40**
Cob diameter (CD)	**0.39**	0.15	**-0.32**
Number of kernels per row (NK)	**0.35**	**-0.48**	0.27
Kernel thickness (KT)	0.20	**0.44**	0.24
Number of rows per ear (NR)	0.23	-0.23	**-0.61**
Ear weight (EW)	**0.35**	0.11	0.07
Cob weight (CW)	**0.43**	-0.06	0.12
Kernel weight (KW)	0.24	**0.53**	**0.30**

$ The bold-faced numbers indicate PC loadings larger than 0.30 and smaller than -0.30 and they were regarded as substantial.

Figure 1.2 Molecular map of 264 $F_{2:3}$ families derived from BH20xBH30.

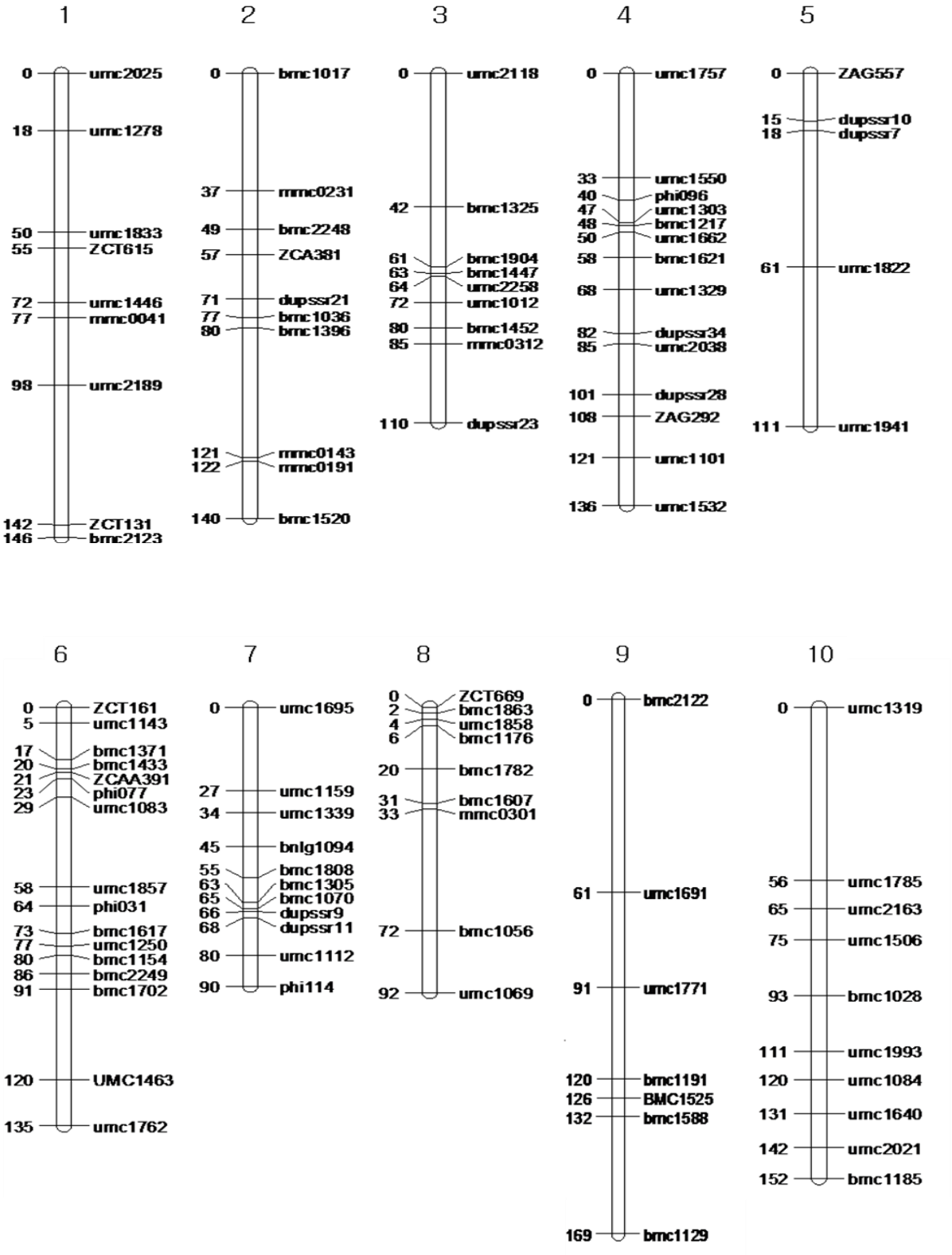


Table 1.6 Parameters associated with QTL for pericarp thickness traits, estimated from 264 F_3 families derived from BH20xBH30 measured in four environments from 2004-2006.

Trait	Bin [#]	QTL position[§] cM	Marker interval	Support interval	LOD	Additive QTL effect[¶]	Partial $R^{2\%}$
Upper Germinal (UG)	1.07	44	umc1278-umc1833	20-54	3.54	2.139	6.0
	2.06	108	bmc1396-mmc0143	92-122	5.32	-2.925	8.9
	3.00	22	umc2118-bmc1325	4-40	5.30	-3.021	9.0
	4.01	26	umc1757-umc1550	8-38	4.21	-2.391	7.5
	4.07	98	umc2038-dupssr28	84-110	3.28	-1.854	5.6
	6.00	0	ZCT161-umc1143	0-6	4.13	-1.691	7.0
	6.05	76	bmc1617-umc1250	72-82	6.04	2.355	10.0
	10.07	130	umc1084-umc1640	22-136	3.26	1.700	5.6
						$R^2_{adj.}$[§§]=33.8	$P_{adj.}$[##]=45.6
Lower Germinal (LG)	2.06	112	bmc1396-mmc0143	98-122	5.04	-3.733	8.4
	3.00	34	umc2118-bmc1325	18-54	4.75	-3.482	8.1
	3.03	80	umc1012-bmc1452	74-86	3.24	2.859	5.5
	4.03	34	umc1550-phi096	14-38	5.12	-3.247	8.6
	4.07	100	umc2038-dupssr28	92-106	6.46	-3.558	10.7
	7.02	38	umc1339-bnlg1094	34-52	3.29	-2.397	5.6
	8.05	8	bmc1176-bmc1782	4-16	4.39	-2.788	7.4
	8.08	46	mmc0301-bmc1056	32-72	3.06	2.987	5.2
	10.07	130	umc1084-umc1640	22-136	3.52	2.362	6.0
						$R^2_{adj.}$[§§]=31.0	$P_{adj.}$[##]=37.9

Table 1.6 Continued.

Trait	Bin #	QTL position§ cM	Marker interval	Support interval	LOD	Additive QTL effect¶	Partial $R^{2\%}$
Upper Abgerminal (UA)	1.10	108	umc2189-ZCT131	92-122	5.80	3.714	9.7
	2.06	104	bmc1396-mmc0143	92-118	7.54	-4.211	12.4
	3.00	14	umc2118-bmc1325	0-28	8.11	-4.332	13.4
	4.06	76	umc1329-dupssr34	58-84	3.95	-2.434	6.7
	9.03	76	umc1691-umc1771	60-92	4.38	-2.959	7.4
						$R^2_{adj.}$§§=29.5	$P_{adj.}$##=36.6
Lower Abgerminal (LA)	1.10	108	umc2189-ZCT131	90-122	6.51	5.567	10.8
	2.06	102	bmc1396-mmc0143	84-118	4.60	-4.946	7.7
	3.00	22	umc2118-bmc1325	6-34	7.46	-7.015	12.4
	3.03	80	umc1012-bmc1452	74-86	2.93	3.402	5.0
	8.04	6	umc1858-bmc1176	4-16	3.89	-2.445	6.6
	9.03	82	umc1691-umc1771	64- 114	3.27	-3.739	5.6
						$R^2_{adj.}$§§=22.9	$P_{adj.}$##=29.5
Crown (CWN)	1.10	106	umc2189-ZCT131	88-122	6.01	2.513	10
	2.06	98	bmc1396-mmc0143	80-114	4.65	-2.427	7.8
	3.00	14	umc2118-bmc1325	0-34	3.79	-2.213	6.5
	4.06	76	umc1329-dupssr34	58-84	2.87	-1.721	4.9
	9.03	82	umc1691-umc1771	70-106	6.74	-2.643	11.1
						$R^2_{adj.}$§§=26.6	$P_{adj.}$##=38.0

Bin number of left flanking marker, taken from Maize GDB. § QTL position from the top of the chromosome as calculated by PLABQTL.

¶ The additive effects of each QTL are calculated as (mean of the BH30 genotypic class – mean of BH20 genotypic class). Therefore, positive values indicate that BH20 carries the allele for decreasing peicarp thickness, and negative values indicate that BH30 contributes the alleles for decreasing pericarp thickness.

% Proportion of phenotypic variation accounted for each QTL calculated by multiple regression in PLABQTL.

§§ Proportion of phenotypic variation explained by the final model. ## Proportion of genotypic variation explained by the final model.

Table 1.7 Parameters associated with QTL for ear traits, estimated from 264 F_3 families derived from BH20xBH30 measured in four environments from 2004-2006.

Trait	Bin [#]	QTL position[§] cM	Marker interval	Support interval	LOD	Additive QTL effect[¶]	Partial $R^{2\%}$
Cob length (CL)	4.05	48	umc1303-bnc1217	46-50	4.08	-0.365	6.9
	9.07	130	bmc1525-bmc1588	120-134	2.87	0.292	4.9
	10.07	144	umc2021-bmc1185	134-152	2.81	0.214	5.4
						$R^2_{adj.}$[§§]=12.3	$P_{adj.}$[##]=19.2
Ear diameter (ED)	1.07	64	ZCT615-umc1446	44-76	2.72	-0.158	4.7
	2.05	72	dupssr21-bmc1036	64-78	6.39	0.636	10.6
	3.04	84	bmc1452-mmc0312	80-96	4.70	-0.142	7.9
	9.04	120	umc1771-bmc1191	106-126	3.61	-0.430	6.1
	10.07	144	umc2021-bmc1185	132-152	3.72	0.507	7.1
						$R^2_{adj.}$[§§]=20.4	$P_{adj.}$[##]=30.9
Cob diameter (CD)	1.08	94	mmc0041-umc2189	86-114	7.22	0.510	11.9
	4.03	34	umc1550-phi096	32-40	7.86	-0.522	12.9
	4.07	98	umc2038-dupssr28	86-110	2.57	-0.208	4.4
	5.05	110	umc1822-umc1941	92-110	4.38	0.320	7.5
	6.00	4	ZCT161-umc1143	0-6	3.67	-0.281	6.2
	6.06	134	umc1463-umc1762	128-134	6.17	-0.437	10.5
	7.03	66	bmc1070-dupssr9	64-70	3.06	0.261	5.2
						$R^2_{adj.}$[§§]=22.7	$P_{adj.}$[##]=36.1

Table 1.7 Continued.

Trait	Bin [#]	QTL position[§] cM	Marker interval	Support interval	LOD	Additive QTL effect[¶]	Partial $R^{2\%}$
Kernel depth (KD)	2.06	112	bmc1396-mmc0143	94-118	5.00	2.005	8.4
	3.04	90	mmc0312-dupssr23	66-100	4.40	-0.289	7.4
						$R^2_{adj.}$[§§]=5.6	$P_{adj.}$[##]=14.8
Number of kernels per row (NK)	2.02	0	bmc1017-mmc0231	0-16	3.67	0.669	6.3
	4.05	52	umc1662-bmc1621	48-60	3.26	-0.734	5.6
	8.05	10	bmc1176-bmc1782	4-22	5.79	0.892	9.6
	10.07	148	umc2021-bmc1185	130-152	2.79	0.307	5.3
						$R^2_{adj.}$[§§]=16.2	$P_{adj.}$[##]=29.1
Kernel thickness (KT)	1.05	2	umc2025-umc1278	0-10	3.19	-0.063	5.5
	3.03	72	umc2258-umc1012	66-80	2.95	-0.055	5.0
	6.06	124	umc1463-umc1762	110-134	3.76	-0.092	6.5
	8.05	8	bmc1176-bmc1782	4-22	3.33	-0.060	5.7
	9.01	0	bmc2122-umc1691	0-26	2.78	0.024	4.7
	10.03	62	umc1785-umc2163	56-76	2.75	-0.050	4.7
						$R^2_{adj.}$[§§]=21.4	$P_{adj.}$[##]=45.4
Number of rows per ear (NR)	1.08	96	mmc0041-umc2189	86-112	5.69	0.252	9.5
	3.04	80	umc1012-bmc1452	72-86	2.72	0.149	4.6
	4.04	42	phi096-umc1303	40-50	3.43	-0.210	5.8
	5.05	110	umc1822-umc1941	88-110	3.95	0.206	6.8
	10.07	122	umc1084-umc1640	112-130	3.45	0.234	5.9
						$R^2_{adj.}$[§§]=22.3	$P_{adj.}$[##]=40.0

Table 1.7 Continued.

Trait	Bin [#]	QTL position[§] cM	Marker interval	Support interval	LOD	Additive QTL effect[¶]	Partial $R^{2\%}$
Ear weight (EW)	2.06	78	bmc1036-bmc1396	70-82	5.22	4.465	8.7
	7.02	68	dupssr9-dupssr11	64-70	4.89	4.590	8.2
						$R^2_{adj.}$[§§]=12.9	$P_{adj.}$[##]=20.2
Cob weight (CW)	3.03	76	umc1012-bmc1452	72-86	4.11	-0.512	6.9
	4.03	34	umc1550-phi096	30-38	6.25	-0.724	10.4
	5.03	0	ZAG557-dupssr10	0-12	4.32	-0.459	7.5
	6.01	20	bmc1371-bmc1433	16-22	3.72	-0.439	6.3
	7.00	0	umc1695-umc1159	0-8	4.55	0.470	7.7
	7.03	78	dupssr11-umc1112	68-86	3.01	0.452	5.1
	8.03	2	ZCA669-bmc1863	0-6	4.62	0.501	7.8
	9.01	60	bmc2122-umc1691	46-72	4.76	-0.534	8.0
						$R^2_{adj.}$[§§]=26.9	$P_{adj.}$[##]=37.1
Kernel weight (KW)	2.02	0	bmc1017-mmc0231	0-20	3.21	-0.600	5.5
	2.06	78	bmc1036-bmc1396	72-82	5.36	0.799	9.0
	5.03	0	ZAG557-dupssr10	0-14	3.26	-0.610	5.7
	6.06	128	umc1463-umc1762	122-134	7.53	-1.211	12.8
	7.00	22	umc1695-umc1159	0-28	3.42	0.629	5.9
	7.02	38	umc1339-bnlg1094	28-46	2.96	-0.725	5.1
	8.08	82	bmc1056-umc1069	72-92	4.85	-0.716	8.2
	9.01	58	bmc2122-umc1691	34-70	4.11	-0.675	7.0
	10.03	62	umc1785-umc2163	56-74	7.80	-0.993	12.9
						$R^2_{adj.}$[§§]=39.6	$P_{adj.}$[##]=56.2

Bin number of left flanking marker, taken from Maize GDB. § QTL position from the top of the chromosome as calculated by PLABQTL.
¶ The additive effects of each QTL are calculated as (mean of the BH30 genotypic class – mean of BH20 genotypic class). Therefore, positive values indicate that BH30 carries the allele for an increase in ear traits, and negative values indicate that BH20 contributes the alleles for an increase in ear traits.
% Proportion of phenotypic variation accounted for each QTL calculated by multiple regression in PLABQTL.
§§ Proportion of phenotypic variation explained by the final model. ## Proportion of genotypic variation explained by the final model.

Table 1.8 Parameters associated with QTL for principal component for pericarp thickness traits, estimated from 264 F_3 families derived from BH20xBH30 measured in four environments from 2004-2006.

Trait	Bin [#]	QTL position[§] cM	Marker interval	Support interval	LOD	Additive QTL effect[¶]	Partial $R^{2\%}$
PC1 for pericarp thickness trait	1.10	112	umc2189-ZCT131	94-126	5.08	1.014	8.5
	2.06	104	bmc1396-mmc0143	90-118	6.41	-1.088	10.6
	3.00	20	umc2118-bmc1325	0-38	4.95	-0.976	8.4
	4.01	28	umc1757-umc1550	6-38	3.84	-0.693	6.9
	4.07	100	umc2038-dupssr28	90-110	3.74	-0.614	6.3
	6.05	76	bmc1617-umc1250	72-82	3.51	0.790	6.0
	9.03	80	umc1691-umc1771	60-118	3.40	-0.695	5.8
						$R^2_{adj.}$ [§§]=29.8	

Bin number of left flanking marker, taken from Maize GDB. § QTL position from the top of the chromosome as calculated by PLABQTL.
¶ The additive effects of each QTL are calculated as (mean of the BH30 genotypic class – mean of BH20 genotypic class). Therefore, positive values indicate that BH20 carries the allele for decreasing peicarp thickness, and negative values indicate that BH30 contributes the alleles for decreasing pericarp thickness.
% Proportion of phenotypic variation accounted for each QTL calculated by multiple regression in PLABQTL.
§§ Proportion of phenotypic variation explained by the final model.

Table 1.9 Parameters associated with QTL for principal components for ear traits, estimated from 264 F_3 families derived from BH20xBH30.

Trait	Bin #	QTL position§ cM	Marker interval	Support interval	LOD	Additive QTL effect¶	Partial $R^{2\%}$
PC1	1.10	106	umc2189-ZCT131	84-126	2.53	0.485	4.4
	2.05	76	dupssr21-bmc1036	62-78	3.73	0.494	6.4
	4.03	36	umc1550-phi096	32-40	8.26	-0.980	13.6
	5.03	0	ZAG557-dupssr10	0-8	4.37	-0.596	7.6
	6.06	134	umc1463-umc1762	128-134	5.33	-0.745	9.2
	7.00	0	umc1695-umc1159	0-8	4.79	0.608	8.2
	7.02/03	74	dupssr11-umc1112	68-90	2.57	0.553	4.4
	9.03	62	umc1691-umc1771	50-74	2.57	-0.446	4.4
	10.07	144	umc2021-bmc1185	136-152	3.53	0.493	6.8
							$R^2_{adj.}$ §§= 32.7
PC2	1.05	4	umc2025-umc1278	0-12	3.88	-0.409	6.6
	2.02	0	bmc1017-mmc0231	0-16	4.73	-0.392	8.1
	4.06	64	bmc1621-umc1329	58-74	5.22	0.496	8.8
	4.08	108	dupssr28-ZAG292	102-114	3.90	-0.350	6.6
	6.06	134	umc1463-umc1762	124-134	4.38	-0.397	7.6
	8.05	12	bmc1176-bmc1782	6-24	7.33	-0.507	12.1
	9.07	124	bmc1191-bmc1525	120-134	2.94	-0.301	5.1
							$R^2_{adj.}$ §§= 36.6
PC3	1.08	96	mmc0041-umc2189	86-116	5.34	-0.407	9.0
	2.07/08	124	mmc0191-bmc1520	120-136	3.03	-0.353	5.6
	3.03	78	umc1012-bmc1452	72-82	5.86	-0.536	9.8
	3.04	96	mmc0312-dupssr23	88-110	3.48	0.598	5.9
	5.05	104	umc1822-umc1941	88-110	6.40	-0.576	10.8
	6.05	114	bmc1702-umcc1463	100-126	4.43	-0.590	7.6
	8.04	4	bmc1863-umc1858	0-6	2.67	-0.006	4.6
	10.07	124	umc1084-umc1640	112-132	2.67	-0.314	4.6
							$R^2_{adj.}$ §§=33.3

Bin number of left flanking marker, taken from Maize GDB. § QTL position from the top of the chromosome as calculated by PLABQTL.
¶ The additive effects of each QTL are calculated as (mean of the BH30 genotypic class – mean of BH20 genotypic class).
% Proportion of phenotypic variation accounted for each QTL calculated by multiple regression in PLABQTL.
§§ Proportion of phenotypic variation explained by the final model.

Table 1.10 List of common QTL intervals associated with pericarp thickness and ear architecture traits and corresponding PC-QTL estimated from 264 F_3 families derived from BH20xBH30.

Chromosome	Common QTL interval (cM)	Traits	PC-QTL (cM)
Pericarp thickness traits			
1	106-108	UA, LA, CWN	112%
2	98-112	UG, LG, UA, LA, CWN	104
3	14-34	UG, LG, UA, LA, CWN	20
3	80	LG, LA	
4	26-34	UG, LG	28
4	76	UA, CWN	
4	98-100	UG, LG	100
8	6-8	LG, LA	
9	76-82	UA, LA, CWN	80
10	130	UG, LG	
Ear architecture traits			
1	94-96	CD, NR	96 (PC3)
2	0	NK, KW	0 (PC2)
2	72-78	ED, EW, KW	76 (PC1)
3	72-90	ED, KD, KL, NR, CW	78 (PC3)
4	34-52	EL, CD, NK, NR, CW	36 (PC1)
5	0	CW, KW	0 (PC1)
5	110	CD, NR	104 (PC3)
6	124-134	CD, KL, KW	134 (PC1, PC2)
7	66-78	CD, EW, CW	
8	2-10	NK, KL, CW	4 (PC3) 12 (PC2)
9	58-60	CW, KW	62 (PC1)
9	120-130	EL, ED	124 (PC3)
10	62	KW, KW	
10	144-148	EL, ED, NK	144 (PC1)

% The peak of PC-QTL position located within the common QTL interval as calculated by PLABQTL.
UG: Upper germinal, LG: Lower germinal, UA: Upper abgerminal,
LA: Lower abgerminal, CWN: Crown.
CL: Cob length, ED: Ear diameter, CD: Cob diameter, KD: Kernel depth, NK: Number of kernels/row, KT: Kernel thickness, NR: Number of rows/ear, EW: Ear weight, CW: Cob weight,
KW: Kernel weight.

CHAPTER TWO

MARKER ASSISTED PYRAMIDING OF FAVORABLE PERICARP THICKNESS ALLELES TO IMPROVE WAXY CORN LINES

ABSTRACT

Marker assisted selection (MAS) may be useful for validating QTL effects and pyramiding favorable alleles in a fresh waxy corn breeding program. From the previous QTL mapping study, we found nine promising QTL regions for pericarp thickness with favorable alleles associated with thinner pericarp coming from both inbred parents, BH20 and BH30. A marker assisted selection population was derived from crosses between mapping population $F_{2:3}$ families according to favorable phenotypes and favorable pericarp QTL alleles. The objectives in this study were to (1) pyramid favorable alleles for thinner pericarp, while maintaining favorable ear traits, and then examine improvement of these traits, (2) evaluate genetic relationships among traits in MAS population, and (3) validate QTL effects detected in the initial QTL study.

Thinner pericarp was observed in all kernel regions in the MAS population in comparison to that of the $F_{2:3}$ mapping population grown previously in different years, but the MAS population showed a wider range of values. For most of the pericarp and ear traits, correlation and principal component analysis (PCA) results for the MAS population showed results similar to the $F_{2:3}$ mapping population. Notably, most pericarp thickness traits were negatively correlated with most ear traits in MAS population except for cob diameter, kernel thickness, and cob weight. At least one marker from all of the selected pairs of markers flanking the nine pericarp thickness QTL showed a significant association with one or more pericarp thickness traits in MAS

population. Pyramiding significant favorable marker alleles showed reduction of pericarp thickness on all kernel regions. Comparing groups of lines in the MAS population sorted by: 1) phenotypes for thinner pericarp; 2) favorable QTL alleles for pericarp thickness; and 3) unfavorable alleles for pericarp thickness, we found that the marker based selection showed some potential to be as effective as the phenotypic selection for reducing pericarp thickness. Therefore, the QTL information for pericarp thickness traits and the MAS results could be useful for introgression of favorable loci into more adapted and productive U.S. germplasm with waxy endosperm.

INTRODUCTION

Kernel pericarp thickness and ear architectural traits are important selection criteria in fresh waxy corn breeding programs as they are associated with consumer sensory and visual preference. Pericarp thickness is associated with tenderness of fresh consumption waxy and sweet corn, drying rate of shelled corn, and popping expansion of popcorn (Ito and Brewbaker, 1981; Mohamed et al. 1993; Stroshine et al. 1987). Ear traits such as longer and larger ear size and larger edible portion are generally preferred by consumers. Therefore, thin pericarped waxy corn with favorable ear traits may appeal to consumers and help in introducing fresh waxy corn to the U.S. market.

Studies on pericarp thickness of dent corn, popcorn and sweet corn have been performed, but to our knowledge, there are no similar genetic studies on fresh waxy corn. Similarly, there have been studies on ear architecture for dent corns but no published studies on waxy corns. Since many Asian waxy corn varieties have poor agronomic performance yet score highly for

consumer preference and taste traits, understanding more about the genetic basis of taste traits will provide information useful to breeding fresh waxy corn with good consumer desirability in germplasm adapted to the U.S. with good agronomic performance.

In chapter one, we discussed results from analysis of the $F_{2:3}$ mapping population derived from a cross between South Korean inbreds BH20 and BH30, which were bred for taste quality and combining ability in their F_1. Average pericarp thickness of the $F_{2:3}$ population was similar to the midparent value of the parental inbreds. Some lines in the population showed transgressive segregation, and QTL effects for reducing pericarp thickness came from both parents. Therefore, it was postulated that thinner pericarp could be achieved through pyramiding favorable alleles from both parents in a selection program. The five kernel regions where pericarp thickness was measured were highly correlated, and most of the QTL for the different kernel regions for pericarp thickness mapped to similar chromosome regions correspondingly. Principal component analysis (PCA) results indicated that the first principal component (PC) explained most of the phenotypic variation of the five pericarp thickness traits. Therefore, pericarp thickness of different kernel regions appeared to be under common genetic control.

Various ear traits were measured, and relationships between these traits and pericarp thickness traits were determined in the $F_{2:3}$ mapping population. Notably, cob diameter and cob weight were weakly positively associated with all five pericarp thickness traits (Table 1.4). Four pericarp thickness traits were also weakly positively associated with kernel thickness ranging from $r=0.14$ ($P<0.05$) to $r=0.19$ ($P<0.01$) (Table 1.4). These results may be due in part to general parental linkage effect since BH30 had thinner pericarp and showed smaller kernel and cob size than BH20. However, these relationships may be unfavorable in breeding programs as it is desirable to achieve thinner pericarped waxy corn lines without diminishing favorable ear traits

such as cob diameter, cob weight, kernel depth, and kernel thickness. Favorable alleles for improving ear architecture came from both parents. Through PCA, we grouped ear traits into three sets of meaningful PCs. Phenotypic and genetic evaluation of ear traits on the population showed the possibility of improvement in overall ear size and desirable ear architecture traits in materials derived from this population.

Information provided by marker-trait associations can be used to improve breeding progress through the use of marker assisted selection (MAS) for desired alleles to increase genetic gain from selection. Many studies have tested and discussed the effectiveness of MAS through simulations and experiments (Zhang and Smith, 1992; Hospital and Charcosset, 1997; Han et al. 1997; Knapp, 1998; Bernardo and Charcosset 2006; Collard and Mackill, 2008). Studies showed that MAS could be more effective than conventional breeding alone because: 1) it can be simpler and more cost effective than phenotypic selection; 2) selection is possible at the seedling stage and before pollination; and 3) a single plant can be selected without the influence of environmental factors (Collard and Mackill, 2008). Given that measuring pericarp thickness is a time consuming, tedious and laborious process, whereas the cost of using molecular markers is becoming less and less expensive, a marker assisted breeding scheme may be an effective method for improving tenderness of fresh waxy corn by assisting development of thinner-pericarped lines. Therefore, MAS using unique Korean germplasm introgressions may enhance breeding for U.S. adapted, high quality tasting and high yielding fresh waxy corn varieties.

One MAS approach that utilizes QTL information for MAS is the pyramiding of favorable alleles to increase likelihood of higher targeted trait value(s). This method has been used in increasing the level of resistance and durability by pyramiding multiple qualitative and quantitative disease resistance genes (Singh et al., 2001; Tabien et al., 2000; Castro et al., 2003).

Creating a population to enable pyramiding of QTL can also enable the validation of QTL effects. One of the risks associated with estimated QTL is that their declaration could be a false positive. Bernardo concluded that the probability of declaring false QTL could be high depending on population sizes, heritability of traits, distances between markers, and sizes of linkage map (Bernardo, 2004). Methods of validating and characterizing QTL include development of near-isogenic lines (NILs) and tests using candidate genes on association panels. Due to the time required in developing NILs, along with having no known genes or a well characterized pathway for pericarp development presently, MAS is a timely and useful method for verifying the effect of QTL, by estimating QTL effects after selection to a subsequent generation and comparison to an earlier generation (Flint-Garcia et al., 2003).

A MAS selection scheme with the goal to produce lines with desirable pericarp and ear traits by incorporating the available information about various phenotypic traits and/or QTL was established. Objectives of this study were (1) to pyramid the favorable alleles for thinner pericarp while maintaining favorable ear traits and then examine the level of improvement of these traits, (2) to evaluate genetic relationships among the traits in MAS population through correlation analysis and PCA, (3) to validate the QTL effects detected in the initial QTL study.

MATERIALS AND METHODS

Selection of Favorable QTL Alleles

Nine QTL marker intervals for pericarp thickness traits were selected from (BH20XBH30) $F_{2:3}$ mapping population. Most of the intervals included two or more individual univariate QTL for the different pericarp regions: upper germinal (UG), lower germinal (LG),

upper abgerminal (UA), lower abgerminal (LA), and crown (CWN) (Figure 1.1). The selected intervals included all seven of the PC-QTL for pericarp thickness traits because they may have pleiotropic effects on reducing overall pericarp thickness of kernel. The PC analysis for pericarp thickness in chapter one explained about 88% of total variation which consisted of overall average effects of five pericarp thickness traits. The seven pericarp PC-QTL explained about 30% of total phenotypic variation of the main principal component. These seven PC-QTL intervals included the five univariate QTL with the highest R^2 and LOD scores for each of the five different pericarp thickness traits. In contrast, QTL5 and QTL6 intervals were selected in spring of 2006 because each QTL had shown significant interactions with three other QTL regions in $F_{2:3}$ mapping population. The interactions were significant when the QTL analysis was performed on the phenotypic data collected from 2004 Illinois nursery, 2005 winter nursery, and 2005 replicated Illinois field. However, no interactions were significant after summer 2006 phenotypic data were included. QTL5 and QTL6 included only single pericarp thickness QTL with relatively small R^2 (Table 2.1).

Seven selected intervals had the favorable allele for thinner pericarp from BH30 and two from BH20, the thicker-pericarped parent (Table 2.1). Selected intervals for QTL3 and QTL4 were both positioned on chromosome 4 with the favorable effect coming from BH30. The QTL5 and QTL9 were both located on chromosome 6 with the favorable effect coming from BH30 and BH20, respectively. The QTL5 was located in the same marker interval in bin 6.00 with QTL for cob diameter in the mapping population. The QTL6 was located in the same marker interval in bin 8.05 with QTL for NK, KT, and PC2 for ear traits from the mapping population. One potential limitation of MAS is the chance of losing linkage between a marker and target locus through recombination. The distance of marker intervals for selected QTL ranged from 4cM to

44cM (Table 2.1). The linkage map we constructed was not in high resolution, and recombination frequencies between QTL and flanking markers varied. Therefore both right and left flanking marker from the map were used to select the QTL interval in most cases. Two flanking markers were selected for seven selected QTL regions. For QTL5 on chromosome 6, and QTL7 on chromosome 9, the QTL peaks were very close to the left flanking marker, so only the left flanking marker was selected for these QTL.

Plant Materials and MAS Procedure

Fourteen families were identified based just on the phenotypic data collected on (BH20XBH30)$F_{2:3}$ ears of seed on F_2 plants self-pollinated in Illinois in 2004; ears of seeds from sib-mated $F_{2:3}$ families grown in Puerto Rico in 2004-2005; and ears of seeds from two replicates of sib-mated F_3 families self pollinated in 2005. The 2006 data was not yet collected at this point in time. These selected families had favorable pericarp thickness traits with large ear size and favorable ear architecture, and were chosen regardless of QTL presence.

Thirty kernels of each selected $F_{2:3}$ family were planted in 2006 at University of Illinois South Farm in paired row plot with two replications. Seven to eight plants in the row were self pollinated to $F_{3:4}$. The two best families (P5 and P6) were selected to be parental lines for developing the MAS population based on agronomic traits, such as fewer tillers, early flowering, and larger ear size (Table 2.2).

Four different $F_{2:3}$ families from the (BH20XBH30)$F_{2:3}$ mapping population were selected to be the parental lines for MAS population based on marker information from QTL estimation. The selected families (P1, P2, P3 and P4) had the most favorable alleles for nine selected QTL intervals (Table 2.2 and Figure 2.1).

In the 2006-2007 winter nursery in Puerto Rico, the $F_{3:4}$ families P5 and P6, selected based on phenotypic traits, were crossed to each of the $F_{2:3}$ families P1, P2, P3, and P4, which were selected based on QTL information. The resultant seeds were planted and F_1 plants self pollinated in a second field in winter nursery to generate F_2 seeds. The F_2 seeds from the eight crosses were planted in paired rows in 2007. Every plant possible was self pollinated. The resultant $F_{2:3}$ ears (MAS population) were planted ear to row in 2008 at University of Illinois South Farm using two-replicate alpha (0, 1) design within the crosses (subpopulations). The respective $F_{2:3}$ parental lines selected from the original mapping population, and the grandparental inbreds BH20 and BH30 were included in the evaluation of each MAS subpopulation.

Each replication of the MAS experiment in 2008 consisted of the eight subpopulations with various population sizes ranging from n = 23 for subpopulation 6 to n = 137 for subpopulation 2 (Figure 2.1), for a total of 493 new $F_{2:3}$ families under evaluation. Thirty kernels of each family were planted per row and thinned to fifteen plants per row after germination. Because of the spring flood, we had to replant 372 rows of the experiment. Four to six plants were self pollinated per row. Because pericarp thickness and ear traits are maternal traits, extra open pollinated ears were harvested to maintain the consistent ear numbers for phenotypic data collection when the number of self pollinated ears was less than five.

Genotypic Data Collection

Thirty random F_3 kernels from $F_{2:3}$ ears of the MAS population were planted in greenhouse and bulk tissues were collected from two week old plants. The tissue was ground to a fine powder using liquid nitrogen and stored at -80℃ before DNA isolation. The DNA was

isolated from ground tissues using the CTAB protocol described by Mikkilineni (1997). A total of 493 families were genotyped for 16 SSR markers selected for QTL (Table 2.1). Since each subpopulation within the overall MAS population was created from a cross between parental lines that were $F_{2:3}$ generation (P1, P2, P3 and P4) and $F_{3:4}$ generation (P5 and P6), genotype frequencies of parental lines were different for each selected QTL (Table 2.2). Therefore, allele frequencies of each locus were expected to be also different in each subpopulation.

Phenotypic Data Collection

Pericarp thickness was measured on five randomly selected F_4 kernels per $F_{2:3}$ family of the MAS population grown in 2008 using a Mitutoyo digital micrometer. Pericarp thickness traits were measured on upper germinal (UG), lower germinal (LG), upper abgerminal (UA), lower abgerminal (LA), and crown (CWN) regions of each kernel using method of Wolf et al. (1969), as modified by Martin, Loesch, and Wiser (1980) (Figure 1.1).

Ear architecture traits were measured on approximately five ears per F_3 familiy. Ear traits were the length of the ear (CL), the number of kernels per row (NK), number of rows per ear at the middle of the ear (NR), the length of ten kernels in the middle of the ear (KT), and diameter of ear before shelling (ED) and of the cob after shelling (CD). The kernel depth (KD) was calculated by subtracting the CD from the ED. The weight of ear (EW) and the cob (CW) and the weight of kernels (TOTALKW) were measured by subtracting CW from EW. In addition to the pericarp thickness and ear traits, day to anthesis (ANT) and severity of tillering rating (TILLERS) where overall severity of tillers in a row is rated from 0 (no tiller) to 9 (more than 3 tillers in every plant) were collected in the field of 2008 for each replication (Table 2.3).

A summary table of the abbreviations used in this chapter is provided in Table 2.3.

Statistical Analysis

All statistical analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC) based on the model: $y_{ijklm} = \mu + \alpha_i + \beta_{j(i)} + \delta_{k(ij)} + \gamma_l + \varepsilon_{ijkl}, + \varphi_{(ijl)m}$, where y_{ijklm} represents the value of individual kernels or ears within a plot, α_i the effect of *i*th replication, $\beta_{j(i)}$ the effect of *j*th subpopulation in the *i*th replication, $\delta_{k(ij)}$ the effect of *k*th block in *j*th subpopulation of *i*th replication, γ_l the effect of the *l*th family, and ε_{ijkl} represents residual error. Since the measurement values were obtained from five random kernels per family per replication for pericarp thickness traits, and from five random ears per family per replication for ear traits, kernels and plants were included in the model as subsample, $\varphi_{(ijl)m}$,to get more precise estimates of means. The analyses were performed using PROC GLM command and least squared means (lsmeans) were generated.

The adjusted means from each replication were pooled together to get a grand adjusted mean. Adjusted means were calculated between two replications and correlations among the traits were estimated. Principal component analysis (PCA) was performed using PROC PRINCOMP to reduce the variables into meaningful principal components (PC) based on the correlation matrix. Eigenvalues (λ), accounting for the proportion of variation attributable to each PC were calculated. Each PC that had λ of above 1 and explained more than 10% of total phenotypic variation was then identified. The PC loadings were examined to identify phenotypic variables with a substantial association with each selected PC. Loadings with absolute values of > 0.3 were judged substantial. The PC scores were then calculated for each family. These PCs were also used as new variables for analysis. The PC analyses are similar to those reported in Upadyayula *et al*., 2006.

The QTL analysis and validation was performed on the overall MAS population and on each subpopulation using single factor analysis. Additive and dominance effects of each marker for the selected pericarp thickness QTL were estimated and tested based on the genotypic and phenotypic associations of the families. Favorable additive effects are decreasing pericarp thickness and increasing ear traits. Since QTL5 and QTL6 for pericarp thickness were located in the same bins with QTL for ear traits in mapping population, single factor analysis was performed on both pericarp thickness and ear traits in MAS population. Single factor analyses were performed on all families (overall MAS population) and on the families within each cross (subpopulation).

To assess the effectiveness of the selection procedure, we compared three groups: the 5% of lines with highest frequency of favorable marker alleles (MOST), the 5% of lines with lowest frequency of favorable marker alleles (LEAST), and 5% of lines with highest PC scores (PS) using PROC GLM.

The single best line from each subpopulation was identified based on pericarp thickness PC scores (phenotypic selection). Similarly, the single best line from each subpopulation was identified based on favorable pericarp thickness QTL alleles (MAS). Eight lines selected using phenotypic selections were compared with the eight lines selected using MAS.

RESULTS

Phenotypic Evaluation

Pericarp thickness traits, upper germinal (UG), lower germinal (LG), upper abgerminal (UA), lower abgerminal (LA) and crown (CWN), measured on the parental families P1, P2, P3,

P4, P5 and P6 in the MAS population in 2008 were generally similar to values for pericarp thickness of the corresponding families in the (BH20xBH30)$F_{2:3}$ mapping population grown from 2004 to 2006 (Table 2.2 and 2.4). The grandparental inbreds, BH20 and BH30, grown in 2008 showed values similar to earlier years.

On average, the overall MAS population in 2008 showed narrower pericarp thickness for the five different regions of kernel in comparison to the overall (BH20xBH30)$F_{2:3}$ mapping population grown in 2004, 2005 winter nursery, 2005, and 2006 (Table2.4). A wider range of pericarp thickness was observed in the overall MAS population than the mapping population, with several outliers for thick pericarp traits in the MAS population. Given that the different populations were grown in different years instead of side by side in the same year, we can not make direct statistical comparisons. Nevertheless, simple observations on the two populations are useful.

The pericarp thickness of all five kernel regions averaged together in MAS population was thinner than the average pericarp thickness of the midparent value of grandparents BH20 and BH30, and it was also similar to the average pericarp thickness of the midparent value of the six selected parental lines, P1, P2, P3, P4, P5 and P6. The average pericarp thickness of each kernel region in overall MAS population was generally narrower than P1, P3 and P4 and was wider than P2, P5 and P6. However, these differences were not statistically significant (Table 2.4 and Figure 2.2).

Subpopulation 5 had the thinnest pericarp in comparison to other MAS subpopulations. The average pericarp thickness of each kernel region for each subpopulation was not significantly different from the midparental value of their corresponding two parents and the grandparental inbreds except for subpopulations 5, 6, and 7, which showed significantly thinner

pericarp than BH20 inbred on LG and LA. However, the average pericarp thickness of five kernel regions for subpopulations 1, 2, 3, 4, and 8 were generally thicker than the corresponding midparent value of their corresponding two parental lines (Table 2.4).

Average ear traits measured for overall MAS population were generally smaller than the traits measured for (BH20xBH30)$F_{2:3}$ mapping population except for CD which was similar and KT which was thicker than the mapping population. Notably, the range of MAS population for each ear trait was wider than the range of the (BH20xBH30)$F_{2:3}$ mapping population (Figure 2.3).

For the ear traits measured on MAS population experiment in 2008, differences among all six parental families were not significant. Differences between the grandparental inbreds BH20 and BH30 were also not significant except for NR. Most parental families showed relatively high values in most ear traits, higher than both grandparental inbreds except for KT and NR. Notably, P2 showed significantly larger values for ED, EW, and TOTALKW than BH30. The P4 line was significantly larger than BH30 for CL, EW and TOTALKW. The P1 line showed larger values than BH30 for CL and EW (Table 2.5).

Most of ear traits in overall MAS population showed more similarity with BH20, which had heavier and longer ears along with larger kernel thickness but fewer number of rows per ear than BH30 (Table 2.5 and Figure 2.3). However, the differences between overall MAS population and each grandparental inbred for most ear traits were not significant, except for significantly larger KT than BH30 and fewer NR than BH30.

The average for ear traits of overall MAS population was more similar to the parental lines selected on phenotypes (P5 and P6) than the parental lines selected on pericarp QTL information (P1, P2, P3 and P4). For example, P2 showed significantly larger values for ED, EW

and TOTALKW than overall MAS population. For NK, P1 and P4 showed larger values than overall MAS population (Table 2.5).

The subpopulation 7 showed relatively larger values for CL, NK, EW and TOTALKW compared to the other subpopulations. However, the differences among the subpopulations were not significant. Most ear traits on subpopulation 7 were larger than the grandparental inbred, BH20 (Table 2.5).

The difference of ANT between the grandparental inbreds, BH20 and BH30, was much larger, about 14 days, when compared to the differences among all six parental lines. Average ANT of MAS population was close to BH20 which showed earlier flowering and was also similar to parental lines. The inbreds BH30 and BH20 had a large difference for TILLERS, with BH30 showing the higher value. Average TILLERS of MAS population was more similar to BH30 than BH20 (Table 2.5).

Correlations and PCA

In the overall MAS population, all five pericarp thickness traits were highly positively correlated, ranging from 0.78 to 0.93. Most ear traits were also significantly correlated at 0.05 probability level. All significant correlations among ear traits were positive except for the negative correlations between KT and most ear traits. The ED, NK, EW, and TOTALKW traits were significantly associated with all of the other ear traits. The CL trait was positively correlated with all ear traits except with KT and NR. The CD trait was also positively correlated with all ear traits except with KD and KT. Notably, KD showed positive associations with every ear trait except CD. The KT trait showed negative correlations with every ear trait except CL, CD and CW. The NR trait showed positive correlations with most ear traits, negative correlation

with KT and no correlation with CL. The CW trait was positively correlated with every ear trait except KT (Table 2.6).

Pericarp thickness traits showed some noteworthy associations with several ear traits. The KT trait was positively correlated with all of pericarp thickness traits. The KD, NK, NR, EW and TOTALKW traits were negatively correlated with all pericarp thickness traits. Four pericarp thickness traits were negatively correlated with ED (Table 2.6).

The TILLERS trait showed no significant correlations with pericarp thickness traits, but ANT showed some weak positive correlations with three pericarp thickness traits. The TILLERS trait showed weak negative correlations with KD, and ANT showed weak negative correlations with CL, NK, NR, EW and TOTALKW. The ANT trait showed weak positive correlations with KT (Table 2.6).

Two separate PCA were performed on five pericarp thickness traits and on eight ear traits. For pericarp thickness traits, one PC explained about 88.6% of total variation with all five pericarp thickness traits showing similar positive substantial loadings. For ear traits, three PCs were selected that explained 78.8% of total phenotypic variation. Ear trait PC1 explained 46.0% of the total phenotypic variation, with positive substantial loadings for all ear traits except KT, which was negative in direction but not with a substantial loading. Ear trait PC2 explained 17.7% of total variation with substantial positive loadings for ED, CD and KT and with substantial negative loadings for CL and NK. Ear trait PC3 explained 15.1% of total variation with substantial positive loadings for CL, KT and CW and with negative loadings for NR (Table 2.7).

QTL Validation

Single factor analysis of variance performed on the overall MAS population detected a number of significant associations between markers and pericarp thickness traits. At least one marker for all QTL intervals was found significant, explaining variation for at least one kernel pericarp thickness region. Notably, markers M01 (QTL1), M02 (QTL1), M04 (QTL2), M11 (QTL6) and M16 (QTL8) were significant for all of five pericarp thickness traits in overall MAS population. The parental contribution of favorable additive alleles for all significant markers followed the results found in the $F_{2:3}$ mapping population except M09. The favorable pericarp thickness QTL alleles came from BH30 for markers from M01 to M12, and from BH20 for M13 to M16. Marker M09 was found to be significant for only LG with favorable thinner effect coming from BH20, but the favorable effect in the mapping population came from BH30. We detected dominance effects of markers M02, M08, M09, and M16 on variation of pericarp thickness traits. Most significant dominance effects observed involved the heterozygous genotype showing thicker pericarp than the mean of the homozygous genotypes. Only the marker M16 exhibited significantly thinner pericarp in heterozygous genotype than mean of homozygous genotypes for LA. The pericarp PC showed significant additive associations with markers M01, M02, M04, M11, M14, and M16, which were also significant with three to five pericarp thickness traits. The markers M02 and M09 were significant for dominance effects for the pericarp PC trait (Table 2.9 and Table 2.12).

Significant additive associations of markers with ear traits were detected. Notably M09 was significant for NK, EW, CW and CL with increasing additive effect coming from BH20. The marker M11 was significant for NK, EW and CL with increasing additive effect coming from BH30 (Table 2.11).

The analysis performed on each subpopulation showed differences with results for the overall MAS population and (BH20xBH30)$F_{2:3}$ mapping population. The total number of traits that were significant for a marker varied among the different subpopulations. For example, M02 was significant for all five pericarp thickness traits for the mapping and the overall MAS population, but was not significant for any pericarp traits in each subpopulation. In contrast, M08 and M10 were not significant for additive effects for any of the pericarp thickness traits in the overall MAS population, but showed significance for most pericarp traits in two and one subpopulations respectively. Subpopulation 2 showed the largest number of markers significant for pericarp traits (Table 2.10 and Table 2.13).

Comparison of analysis of subpopulations with the analysis of overall MAS population showed some inconsistencies in direction of effect on pericarp thickness for parental alleles. For example, the M05, M08, M10, M12, M13, M15 and M16 markers showed a different direction of additive effects for favorable pericarp thickness traits in some subpopulations in contrast to the overall MAS and (BH20xBH30)$F_{2:3}$ mapping populations. The markers M05, M08 and M10 showed a number of significant favorable additive effects for reducing pericarp thickness coming from BH20, in subpopulation 5 for M05, in subpopulation 3 and 4 for M08 and in subpopulation 5 for M10. However, the favorable additive effects for those markers came from BH30 in mapping population. The significant favorable additive effects in subpopulation 2 and 4 for M13 and in subpopulation 8 for M15 came from BH30 instead of BH20, opposite from the results we found from mapping population. The markers M13 and M15 did not show any significant additive effect in overall MAS population.

Markers M12 and M16 showed a difference in their contribution of favorable additive alleles for different subpopulations. The marker M12 showed significant additive effects in

subpopulation 1 and 5. The marker M16 showed significant additive effects in subpopulation 1, 2 and 3. For both markers, favorable additive effects were from BH30 in subpopulation 1. The favorable additive effects were from BH20 in subpopulation 5 for M12, and in subpopulations 2 and 3 for M16. However, the favorable additive effects for M12 came from BH30 in overall MAS and mapping populations and the favorable additive effects for M16 came from BH20 in overall MAS and mapping populations (Table 2.10 and Table 2.13). These inconsistencies may be related to small size of subpopulations and loss of linkage between marker and QTL (Table 2.10 and Table 2.13).

The markers M01, M04, M07, M11 and M14 showed results in subpopulations consistent with results from overall MAS and $F_{2:3}$ mapping populations. However these markers were not significant in all subpopulations (Table 2.10 and Table 2.13). This shows that the overall MAS population could be significant for a marker QTL effect for pericarp thickness traits, but that each individual subpopulation may not be significant

The markers M02 and M06 were not significant for additive effects in all of the individual subpopulations. However M02 showed dominance in subpopulation 8, exhibiting significantly thicker pericarp in heterozygous lines than the average of homozygous lines for favorable and unfavorable alleles. The marker M06 also showed significant dominance in subpopulation 2 with a significantly thinner pericarp in heterozygous lines than the average of homozygous lines (Table 2.10 and Table 2.13).

MAS Effectiveness and Selection

Two methods were used to select the best favorable thin-pericarped lines from the MAS population. The first method was phenotypic selection (PS) based on PC scores for pericarp

thickness traits, with the top 5% of lines from MAS population selected. The second method was marker score selection calculated by summing the number of favorable alleles of the 16 marker genotypes, with the top 5% of lines (MOST) and bottom 5% of lines (LEAST) selected. Comparison of these three groups of lines showed the PS group had significantly thinner pericarp for all kernel regions and lower pericarp PC scores than both the MOST and LEAST groups. The PS group showed on average thinner pericarp than BH30, the thinner grandparental inbred, whereas the MOST group exhibited similar pericarp thickness to BH30. The MOST group had significantly thinner pericarp than the LEAST group in all five pericarp thickness traits and PC scores. The MOST group had wider range of pericarp thickness among lines (PC scores -3.51 to 0.36) than the PS group (PC scores -4.58 to -2.59) (Figure 2.4). There were no significant differences among the three groups for most ear traits, but we found the PS group had a larger value than the LEAST group for NR, and that the MOST group had larger value than the LEAST group for TOTALKW (Table 2.14).

For each MAS subpopulation, the line with the highest percentage of favorable alleles for selected pericarp QTL (MAS), and the line with the lowest pericarp PC score (PS), were selected (Table 2.15). The average PC score for the eight selected lines with the lowest PC scores in each subpopulation had a smaller value than the eight lines selected based on largest number of favorable alleles. However this was not consistent for all individual populations. For example, line 107-4, selected based on PC score and 106-2, selected based on percent favorable alleles, both from subpopulation 6, showed similar low PC scores, -3.51 and -3.50, respectively (Table 2.15).

The selection process produced detectable results. Half of the selected lines which had the largest number of favorable alleles, 100-6 (subpopulation 5), 15-2 (subpopulation 1), 106-2

(subpopulation 6) and 48-1 (subpopulation 2), showed smaller PC scores than both grandparental inbreds (BH20 = 3.00 and BH30 = -1.78), and parental lines (P1=0.06, P2=-0.51, P3= 1.72, P4= 1.22, P5= -1.73 and P6= -1.32) (Table 2.8 and Table 2. 16).

When pericarp thickness of five kernel regions and PC scores were plotted by percentage of favorable significant QTL alleles on overall MAS population, decreasing pericarp thickness and PC scores were observed by increasing the amount of favorable alleles (Figure 2.5). The R^2 for the plots were relatively similar for all pericarp thickness traits and PC scores, ranging from 8.53% for lower abgerminal to 10.79% for pericarp PC scores (Figure 2.5).

DISCUSSION

Previous QTL analyses for pericarp thickness and ear traits (Chapter One) provided useful background information for potential improvement of fresh waxy corn germplasm through MAS. Through selecting lines with favorable QTL alleles and crossing them to lines with favorable phenotypic traits, we created MAS population consisting of eight subpopulations designed to validate and pyramid QTL while maintaining favorable ear traits.

Through selection of parental lines with favorable traits and generating a larger population size than the mapping population, the overall MAS population showed decreased means compared to the mapping population and also showed broader ranges for pericarp thickness traits. The selected QTL alleles for our MAS study explained only about 30 to 35% of phenotypic variation for pericarp thickness in the mapping population and none of the parental lines selected were fixed for all of favorable alleles. Therefore, all selected QTL for pericarp thickness had segregating favorable and unfavorable alleles in $F_{2:3}$ MAS population, and about

65% of the phenotypic variation was not explained by selected QTL. Genetic effects not explained by selected QTL likely contributed to the phenotypic variation in the overall MAS population. This may have also contributed to relatively thicker pericarp averages in each subpopulation compared to the corresponding midparent values.

The average values for ear traits in overall MAS population were smaller than for the mapping population and parental families. It may be due to the environmental effect of poor weather condition in 2008. Although there was a general decrease in values for the ear traits, most of the ear traits in MAS population showed more similarity with BH20, which had bigger ear and kernel size and length than BH30. Although significant reductions were detected in most ear traits, wider ranges of ear traits were also observed in MAS population than the $F_{2:3}$ mapping population, so there may be opportunity to select lines with better ear traits (Figure 2.3).

High correlations among pericarp thickness traits were found in MAS population, similar to the mapping population. For PCA results, the evidence of high correlation among pericarp thickness traits was clear in MAS population as just one PC needed to be selected to explain 88.6% of overall pericarp thickness variation. This result supports our finding from mapping population in that pericarp thickness throughout the kernel is largely under common genetic control, despite differences in absolute thickness for different kernel regions.

More significant correlations were found among ear traits in MAS population than the mapping population. Most ear traits showed significant positive correlations in both MAS population and mapping population, with a few exceptions. The KT trait showed significant negative correlations with every ear trait except CL, CD and CW in MAS population (Table 2.6). The KT trait showed significant positive correlations with CL, ED, CD, CW and KW in mapping population (Table 1.4). The correlation matrix of ear traits in MAS population may indicate that

KT functions in opposite direction with the other ear traits because it represents kernel thickness whereas the other traits, such as ED, KD, NK, NR, EW and TOTALKW, are related in increasing the overall ear size and kernel number. Only the traits associated with cob characteristics, CL, CD and CW, did not show significant correlations with KT. This result may be due to the parental effects of BH30 which had significantly smaller kernel size but larger kernel number than BH20.

The pericarp PCA results in MAS population were similar to PCA for the mapping population in which all five traits were reduced into a single PC with similar high loadings. Ear trait PCA for the MAS population was different from PCA for the mapping population. This result may be useful for understanding meaningful relationships among the ear traits in MAS population. Three PCs were selected for ear traits, explaining 78.8% of total variation in MAS population. Ear PC1 had positive substantial loadings for all ear traits except KT, explaining 46.0% of total phenotypic variation. This implies that most ear traits measured were highly positively correlated, and were the major traits explaining a large amount of total phenotypic ear variation. The PCA result for the MAS population was somewhat similar to the PCA result for the mapping population in that all ear traits except KT, NR and KW showed substantial loadings in mapping population.

Ear PC2 in MAS population had positive substantial loadings for ED, CD and KT and negative substantial loadings for CL and NK, explaining 17.7% of total phenotypic variation. This is in contrast to PC1 where CL and NK had positive substantial loadings. The MAS population showed substantial loadings for ED and CD, whereas the mapping population did not.

Ear PC3 had positive substantial loadings for CL, KT and CW and a negative substantial loading for NR, explaining 15.1% of total phenotypic variation. This result indicated that longer

cob, thicker kernel, and heavier cob were associated with the width development of ear. Ear PC3 in mapping population showed negative association of CL and KW with ED, CD and NR. In the mapping population thin pericarp was correlated with thin cob diameter and weight, but in the MAS population these associations became weaker. Thin pericarp in MAS population was associated with thicker ear, more kernel depth, more kernels in row, more kernel rows, heavier ear, and heavier kernels even though thin pericarp was associated with smaller kernel thickness. By making crosses of lines selected based on ear phenotype with lines selected based on genotype for pericarp QTL, and then generating a new population, we were able to produce some new favorable associations between pericarp thickness and ear traits.

Agronomic problems with the germplasm used for this study include late flowering and tillering. The ANT trait showed some negative association with ear traits and some positive association with three pericarp thickness traits in this germplasm background, and these relationships might be unfavorable for fresh waxy corn hybrid breeding. However, the correlations were relatively small and the overall MAS population showed earlier flowering than the late flowering grandparental inbred, BH30. Therefore, reducing ANT may be feasible through further breeding efforts for thin pericarp and favorable ear development.

The overall MAS population showed significant thinner pericarp thickness effects of M01 (QTL1), M02 (QTL1) and M04 (QTL2), similar to the results found from mapping population. These markers were significantly associated with controlling all five kernel regions for pericarp thickness and the pericarp PC in both MAS and mapping populations. This result indicates that QTL1 and QTL2 are important loci that were associated with controlling overall pericarp thickness regions. Notably, when the single factor analyses were performed within each subpopulation, M02 showed no significant associations, whereas M01 and M04 were significant

within a number of subpopulations. Small population size may be a reason for different results in each subpopulation in comparison to the overall MAS population. Marker M03, a flanking marker to QTL2, was not significant in overall MAS population, which may indicate loss of linkage between the QTL and marker.

Markers M05 (QTL3) and M07 (QTL4) were significant for two pericarp thickness traits in overall MAS population, but we did not observe significant associations of these markers with the pericarp PC, which we previously found in mapping population. This result may indicate that these QTL have relatively small effects and may not have detectable pleiotropic effects on overall pericarp thickness traits in the MAS population. Markers M06 and M08, the other flanking markers to QTL3 and QTL4, were not significant in overall MAS population, which again may indicate loss of linkage between QTL and marker.

There were other trends in significance patterns between the mapping and MAS populations. The M11 (QTL6) marker was significant for only one pericarp thickness trait in mapping population, but was significant for all five pericarp thickness traits and pericarp PC in overall MAS population. This result shows that by creating the new generation of MAS population, we were able to detect QTL with pleiotropic effects on pericarp thickness traits that were not detectable in the mapping population context. In contrast, M12 (QTL7) was significant for only one pericarp thickness trait in overall MAS population whereas it was significant for three pericarp thickness traits and PC in the mapping population. The markers M14 (QTL8) and M16 (QTL9) were significant for multiple pericarp thickness traits and PC in MAS population, similar to the results from mapping population.

Single factor analyses on the subpopulations showed some results where the parental allele with the favorable effect was different between some subpopulations and the overall MAS

and mapping populations. Notably, M05 (in subpopulation 5), M08 (in subpopulation3 and 4), M10 (in subpopulation 5), M12 (in subpopulation 1), M13 (in subpopulation 2 and 4), M15 (in subpopulation 8) and M16 (in subpopulation 1) showed favorable reducing pericarp thickness effects opposite from overall MAS and mapping populations. This result may be due to the small population sizes of subpopulations which led to sampling effects, the low genetic frequency of homozygous favorable alleles at the loci, and recombination of markers and QTL l. These factors influence reliable estimation of QTL-marker association effects. Therefore, while the overall MAS population result appears more reliable due to the large population size, further investigation involving certain subpopulations would be necessary, particularly if breeding selections were to be made.

A number of ear traits also showed significant associations with the markers, but the effects were small. We selected M09 (QTL5) and M11 (QTL6) initially due to the association between the selected pericarp QTL and the QTL for some ear traits. However these markers showed larger associations with some ear traits than any other marker in the MAS population. The markers M09 and M11 showed additive effects for increasing ear trait values, coming from BH30 and BH20 parental alleles, respectively. This likely is due to associations of different linked genes. Because the favorable allele for M11 for reducing pericarp thickness comes from BH30 and the unfavorable allele for ear traits comes from BH30, we likely would want to try to break the linkage between pericarp thickness associated QTL and ear trait associated QTL for M11. This would achieve linked favorable alleles for both pericarp and ear traits for breeding efforts.

Overall, single factor analysis results on MAS population validated that nine markers, M01 (QTL1), M02 (QTL1), M04 (QTL2), M05 (QTL3), M07 (QTL4), M11 (QTL6), M12

(QTL7), M14 (QTL9) and M16 (QTL8), were highly significant for controlling multiple pericarp thickness traits. The other seven markers that we initially selected for MAS did not show significant additive effects or only showed dominance effects. The results for these seven markers did not follow the mapping population QTL results. This may be due to the low marker density resulting loss of linkage between marker and QTL. However, because we selected both flanking markers for the QTL, and at least one marker per QTL showed significant additive effect on reducing pericarp thickness, all selected QTL were shown significant for pericarp thickness traits in the MAS population.

By comparing percentages of favorable alleles in MAS population with pericarp thickness phenotypic data, we were able to find that increasing number of favorable alleles tends to decrease pericarp thickness in all kernel regions and pericarp PC (Figure 2.5). Because the MAS population was a relatively small population of new $F_{2:3}$ families, derived from $F_{2:3}$ families of the mapping population which were not homozygous for all favorable QTL marker alleles, we did not find new $F_{2:3}$ families with favorable homozygous genotypes at all selected QTL. However, the trend of the box plots in Figure 2.5 for the range of frequencies observed for favorable alleles, showed that pyramiding favorable QTL for pericarp thickness was effective in reducing pericarp thickness.

The strategy to develop the MAS population involved selecting lines based on favorable ear phenotype and crossing with lines selected for favorable genotypes.for pericarp thickness. Thus it was not surprising that smaller means for pericarp thickness were observed in the PS group, comprised of lines identified just on phenotypic PCA scores, in comparison to that for the MOST group, comprised with lines identified just on favorable genotypes for pericarp thickness QTL. Importantly, due to the smaller means and broader ranges for pericarp thickness traits of

the MOST group in comparison to the LEAST group, we conclude that markers were very effective in reducing pericarp thickness.

Comparison of the line with best favorable phenotype for thin pericarp from each subpopulation and the line with largest number of favorable QTL alleles from each subpopulation revealed that the lines with best phenotypes showed thinner pericarp than lines with largest number of favorable alleles. This result is not surprising, and is likely due to the other genetic factors that the identified QTL did not explain and/or due to environmental effects. Most selected lines with largest number of favorable alleles, however, had a few loci that were homozygous with unfavorable alleles and also had a few selected loci that were heterozygous. Pericarp thickness and PC scores vary largely among the lines selected for the largest number of favorable QTL alleles. Fixing the loci to be homozygous with favorable alleles, or introgressing and pyramiding more favorable alleles by crossing with the best genotypic and phenotypic lines may further reduce pericarp thickness in future generations. Lines 100-6, 15-2, 106-2 and 48-1, with most favorable QTL alleles, showed thinner than pericarp values than the grandparental inbreds and all parental lines. This suggests that pyramiding QTL favorable alleles can be effective in reducing pericarp thickness, in conjunction with normal phenotypic segregation and selection.

This study validated and confirmed the QTL results from the mapping population. One of the problems of QTL analysis is the result may vary with different population sizes such that small population size leads to overestimating the real QTL effect and underestimating QTL number and interactions (Melchinger et al., 1998). Therefore, demonstrated reproducibility of the marker-QTL associations across populations, generations and environments is required for these markers to be useful in practical breeding programs (Dudley, 1993). Our study of a new

population in a different environment validated several promising markers that could be used in a further MAS program. We were able to generate improvements in the means of the overall MAS population relative to parental and grandparental lines, and to identify new lines with significantly thinner pericarp while maintaining the favorable ear traits. Therefore, the best lines we identified could be: 1) crossed to each other and selections in subsequent segregating generations made to further improve means, or 2) used as donor lines in backcross programs for introgression of favorable alleles into more adapted U.S. lines to create germplasm useful in producing better waxy corn hybrids.

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TABLES AND FIGURES

Table 2.1 List of QTL, marker information and related traits.

	Bin	Marker distance (in cM)	Left flanking marker	Right flanking marker	Favorable alleles	Pericarp regions
QTL1	2.06	41	bmc1396 M01	mmc0143 M02	BH30	UG (8.9)†, LG (8.4), UA (12.4), LA (7.7), CWN (7.8), PC (10.6)
QTL2	3.00	42	umc2118 M03	bmc1325 M04	BH30	UG(8.9), LG (8.1), UA (13.4), LA (12.4), CWN (6.5), PC (8.4)
QTL3	4.01	33	umc1757 M05	umc1550 M06	BH30	UG (7.5), PC (6.9)
QTL4	4.07	16	umc2038 M07	dupssr28 M08	BH30	UG (5.6), LG (10.7), PC (6.3)
QTL5	6.00		ZCT161 M09		BH30	UG (7.0)
QTL6	8.05	14	bmc1176 M10	bmc1782 M11	BH30	LG (7.4)
QTL7	9.03			umc1771 M12	BH30	UA (7.4), LA (5.6), CWN (11.1), PC (5.8)
QTL8	1.10	44	umc2189 M16	ZCT131 M13	BH20	UA (9.7), LA (10.8), CWN (10.0), PC (8.5)
QTL9	6.05	4	bmc1617 M14	umc1250 M15	BH20	UG (10.0), PC (6.0)

† Proportion of phenotypic variation accounted for QTL.
UG: Upper germinal, LG: Lower germinal, UA: Upper abgerminal, LA: Lower abgerminal, CWN: Crown, PC: Principal component for pericarp thickness traits.

Table 2.2 Phenotypic and genotypic information of selected parental lines measured on (BH20xBH30)$F_{2:3}$ mapping population[#].

	By phenotype		By genotype						
	P5 (112)	P6 (143)	P1 (44)	P2 (149)	P3 (154)	P4 (189)	BH20	BH30	(BH20XBH30) F1
M01[##]	H	B	B	B	H	H	A	B	H
M02	B	B	H	B	H	H	A	B	H
M03	B	H	H	H	H	H	A	B	H
M04	A	A	H	B	H	-	A	B	H
M05	H	A	H	B	B	H	A	B	H
M06	B	B	H	B	B	H	A	B	H
M07	H	A	H	H	B	B	A	B	H
M08	H	H	H	H	B	B	A	B	H
M09	B	H	H	A	H	A	A	B	H
M10	H	H	H	H	-	H	A	B	H
M11	H	H	H	H	H	H	A	B	H
M12	A	H	B	H	B	H	A	B	H
M16	B	H	H	H	H	H	A	B	H
M13	B	A	H	H	H	A	A	B	H
M14	B	A	H	H	H	H	A	B	H
M15	A	A	H	A	H	B	A	B	H
Pericarp thickness traits									
UG	35.2±2.3[%]	33.0±1.9	51.6±8.7	44.7±1.6	52.4±1.7	46.2±4.1	58.6±2.9	45.9±0.6	57.0±2.3
LG	41.7±3.5	37.9±2.0	61.0±7.5	58.8±0.8	64.7±2.0	57.3±3.8	73.0±2.2	53.3±1.1	64.6±2.8
UA	36.4±3.0	33.5±2.1	44.1±4.2	46.2±1.9	50.6±0.6	45.1±3.9	64.6±1.6	34.0±1.3	49.1±2.5
LA	40.9±2.7	38.9±2.3	55.7±5.9	57.2±1.7	69.7±1.6	54.4±4.0	91.6±0.6	38.1±1.5	62.2±3.4
CWN	30.9±3.4	27.4±1.7	32.2±3.2	31.3±2.2	36.7±1.2	37.7±3.9	46.7±1.7	31.4±0.7	35.8±1.8
Average	37.0±2.8	34.1±2.0	48.9±5.8	47.6±1.2	54.8±1.2	48.1±3.8	66.9±1.6	40.5±0.9	53.7±2.4
Ear traits									
CL	14.4±0.7	15.4±0.7	14.8±0.7	13.8±0.9	14.0±1.3	15.2±1.0	-	-	-
ED	32.2±1.4	35.1±0.9	35.2±1.8	37.8±1.6	34.0±2.6	33.7±1.2	-	-	-
CD	19.8±0.7	20.8±1.0	20.4±0.8	22.7±0.6	21.0±0.4	19.7±0.8	-	-	-
KD	12.1±2.4	14.1±2.4	14.3±1.9	14.6±1.8	13.0±3.2	13.8±2.1	-	-	-
NK	26.5±1.5	30.5±0.6	27.4±1.7	28.5±0.6	28.4±1.3	31.3±0.4	-	-	-
KT	5.1±0.3	5.0±0.2	5.0±0.2	4.8±0.2	4.7±0.1	4.9±0.4	-	-	-
NR	10.2±0.2	11.3±0.3	10.2±0.1	11.7±0.4	11.1±0.4	11.5±0.4	-	-	-
EW	67.7±17.3	101.1±23.9	71.0±7.0	92.0±28.0	63.7±26.3	80.8±19.2	-	-	-
CW	6.7±0.5	10.3±1.4	8.5±0.9	8.9±1.7	9.5±0.9	8.3±0.9	-	-	-
KW	23.6±1.2	25.0±1.2	23.1±0.2	22.6±0.9	19.2±2.0	20.6±0.5	-	-	-

Genotype was measured from $F_{2:3}$ mapping population and phenotype were measured from the population in 2003-2006.

From M01 to M11, the favorable alleles for reducing pericarp thickness is B and from M12 to M16, the favorable alleles for reducing pericarp thickness is A.

% Standard errors are attached.

UG: Upper germinal, LG: Lower germinal, UA: Upper abgerminal, LA: Lower abgerminal, CWN: Crown, CL: Cob length, ED: Ear diameter, CD: Cob diameter, KD: Kernel depth, NK: Number of kernels/row, KT: Kernel thickness, NR: Number of rows/ear, EW: Ear weight, CW: Cob weight, KW: Mean weight of 100 kernels.

Figure 2.1 MAS population development.

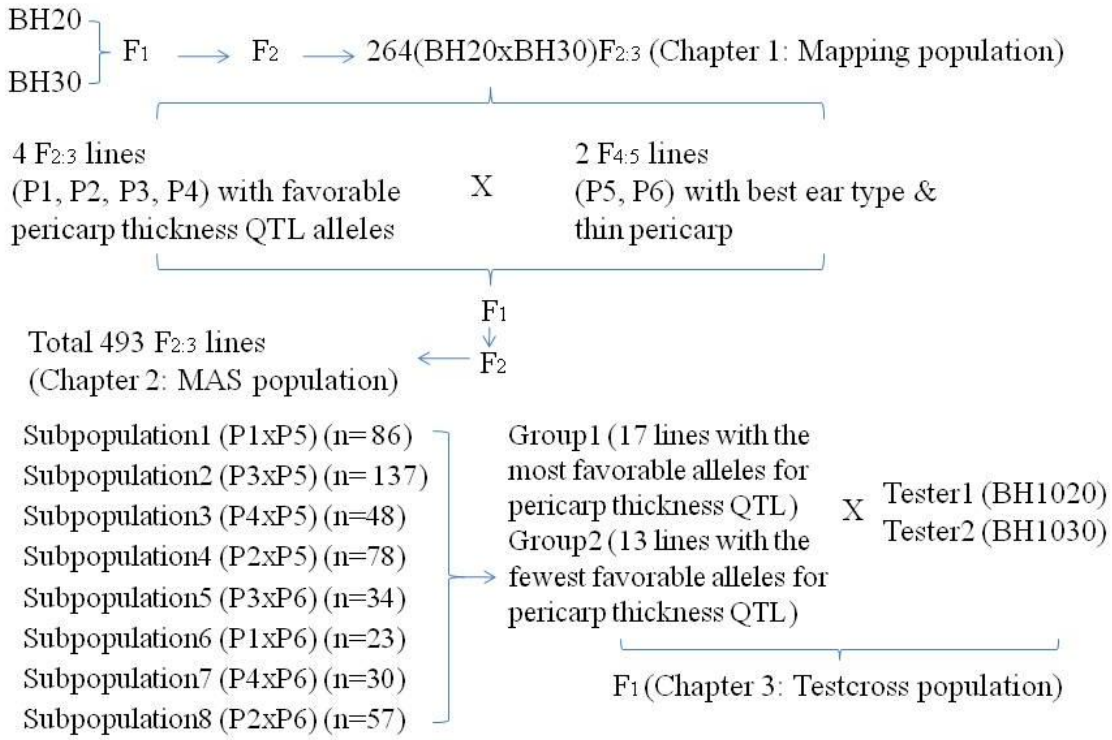


Table 2.3 List of phenotypic trait measurements in MAS population.

Trait	Abbreviation	How measured/calculated
Pericarp thickness traits		
Upper germinal	UG	Thickness of upper germinal region of pericarp in µm
Lower germinal	LG	Thickness of lower germinal region of pericarp in µm
Upper abgerminal	UA	Thickness of upper abgerminal region of pericarp in µm
Lower abgerminal	LA	Thickness of lower abgerminal region of pericarp in µm
Crown	CWN	Thickness of crown region of pericarp in µm
Ear inflorescence architecture traits		
Cob length	CL	Length of the cob in mm
Ear diameter	ED	Diameter of the ear before shelled at the middle of the ear in mm
Cob diameter	CD	Diameter of the cob after shelled at the middle of the cob in mm
Kernel depth	KD	Ear diameter - Cob diameter
Number of kernels/ row	NK	Number of kernels of a random row on ear
Number of rows/ ear	NR	Number of rows per ear at the middle of the ear
Kernel thickness	KT	Length of a kernel in mm = Length of ten kernels in cm at the middle of the ear/10
Ear weight	EW	Weight of the ear before shelled in g
Cob weight	CW	Weight of the cob after shelled in g
Total kernel weight	TOTALKW	Ear weight - Cob weight
Agronomic traits		
Days to anthesis	ANT	Planted date-Anthesis date
Tillers	TILLERS	Severity of tillers within a row scored from 0 (no tiller) to 10 (every plant has more than 3 tillers)

Table 2.4a Pericarp thickness trait means of $F_{2:3}$ mapping population measured in four environments from 2004-2006.

		Pericarp thickness traits				
Source	Entries	UG (µm)	LG (µm)	UA (µm)	LA (µm)	CWN (µm)
$F_{2:3}$ mapping population	264	50.73 ± 0.31	66.22 ± 0.35	49.90 ± 0.30	65.30 ± 0.42	37.72 ± 0.23

Table 2.4b Pericarp thickness trait means of grandparents, parental lines, and MAS population followed by each subpopulation measured in 2008.

		Pericarp thickness traits				
Source	Entries	UG (µm)	LG (µm)	UA (µm)	LA (µm)	CWN (µm)
BH20	5	54.86±1.56[§]	81.80±2.65	56.74±1.79	81.69±2.27	42.02±1.22
BH30	5	35.89±1.54	54.14±1.70	32.81±1.05	42.85±2.26	28.17±0.92
P1	2	44.65±3.07	69.05±6.68	40.10±4.63	57.50±7.73	30.70±3.39
P2	2	39.38±2.43	63.39±2.61	40.43±3.08	55.96±1.86	29.03±2.54
P3	2	56.35±1.44	67.85±1.11	49.75±1.50	66.75±1.21	40.20±1.30
P4	2	52.75±1.12	69.85±0.99	46.15±1.34	57.95±1.42	40.45±1.49
P5	4	35.27±2.09	49.73±2.83	35.31±1.55	42.08±1.51	29.72±1.56
P6	4	38.63±1.10	48.90±2.06	37.88±1.28	46.05±0.98	31.58±1.28
MAS population	493	43.87±0.33	59.10±0.41	43.37±0.36	55.60±0.47	35.62±0.29
Subpopulation 1 (P1xP5)	86	45.81±0.83	63.50±0.93	45.47±0.83	60.04±1.11	36.99±0.72
Subpopulation 2 (P3xP5)	137	46.82±0.60	63.81±0.77	46.65±0.64	59.92±0.92	38.46±0.53
Subpopulation 3 (P4xP5)	48	48.68±0.84	63.16±1.07	47.82±1.06	58.66±1.36	40.35±0.81
Subpopulation 4 (P2xP5)	78	40.58±0.79	57.49±0.88	41.44±0.97	54.56±1.18	32.00±0.75
Subpopulation 5 (P3xP6)	34	35.68±0.83	46.98±1.20	34.20±0.93	45.12±1.32	28.44±0.67
Subpopulation 6 (P1xP6)	23	38.43±1.14	50.82±1.54	35.35±1.09	44.32±1.48	29.60±0.94
Subpopulation 7 (P4xP6)	30	40.03±0.87	48.97±0.99	39.72±0.92	47.91±1.09	34.49±0.84
Subpopulation 8 (P2xP6)	57	43.79±0.95	56.56±1.08	42.22±1.01	52.89±1.23	35.24±0.86

§ Standard errors are attached.
UG: Upper germinal, LG: Lower germinal, UA: Upper abgerminal, LA: Lower abgerminal, CWN: Crown.

Table 2.5a Ear and agronomic trait means of $F_{2:3}$ mapping population measured in four environments from 2004-2006.

		Ear traits									
Source	Entries	CL (mm)	ED (mm)	CD (mm)	KD (mm)	KT (mm)	NK	NR	EW (g)	CW (g)	TOTALKW (g)
F2:3 mapping population	264	145.7 ± 0.06	34.30 ± 0.10	21.26 ± 0.05	12.92 ± 0.11	4.87 ± 0.01	28.78 ± 0.12	11.16 ± 0.03	69.78 ± 0.90	9.02 ± 0.08	60.03±0.81

Table 2.5b Ear and agronomic trait means of grandparents, parental lines, and MAS population followed by each subpopulation measured in 2008.

		Ear traits										Agronomic traits	
Source	Entries	CL (mm)	ED (mm)	CD (mm)	KD (mm)	KT (mm)	NK	NR	EW (g)	CW (g)	TOTALKW (g)	ANT (days)	TILLERS (0-10 scale)
BH20	5	135.87±1.83[§]	30.03±1.33	20.24±0.77	9.78±0.87	5.22±0.09	21.57±0.83	8.50±0.28	42.21±1.49	7.80±0.19	34.41±1.33	61.18±0.82	1.55±0.37
BH30	5	103.41±7.35	29.78±1.60	19.93±0.87	9.85±1.26	3.90±0.11	22.85±1.81	13.66±0.93	35.95±5.59	5.00±0.33	30.95±5.35	75.95±1.80	4.64±0.45
P1	2	163.53±5.88	37.13±0.44	22.33±0.83	14.80±0.54	4.56±0.08	31.73±1.18	11.25±0.42	70.99±5.55	9.97±1.11	61.03±4.47	67.50±0.35	5.75±0.48
P2	2	147.88±3.69	41.23±0.51	25.68±1.29	15.55±1.55	4.91±0.14	27.30±1.11	11.03±0.18	78.81±2.43	10.47±0.55	68.34±2.09	67.50±1.14	4.00±0.91
P3	2	144.51±6.53	33.09±0.18	20.55±0.64	12.54±0.60	4.37±0.12	27.08±0.43	10.74±0.12	63.99±3.67	9.83±0.58	54.16±3.39	63.88±0.85	7.25±0.85
P4	2	166.20±2.46	34.31±0.77	21.58±1.02	12.74±0.83	4.69±0.11	32.45±0.57	11.85±0.46	75.85±5.06	10.21±0.75	65.65±4.31	62.63±0.55	6.50±0.65
P5	4	121.73±4.41	31.68±1.11	20.81±0.92	10.87±1.11	4.96±0.10	21.70±1.12	10.23±0.38	41.23±1.73	5.25±0.23	35.98±1.70	60.31±0.62	4.38±0.60
P6	4	143.53±4.16	33.24±0.93	22.96±1.14	10.28±0.70	4.78±0.17	25.28±1.05	11.85±0.18	63.90±1.66	9.17±0.22	54.73±1.51	65.50±0.77	5.63±0.42
MAS population	493	130.12 ± 0.63	30.84 ± 0.14	21.06 ± 0.09	9.78 ± 0.10	5.11 ± 0.02	22.29 ± 0.15	10.03 ± 0.04	45.85 ± 0.45	7.78 ± 0.07	38.10 ± 0.41	65.62±0.20	4.66±0.07
Subpopulation 1 (P1xP5)	86	132.50±1.70	31.26±0.33	21.33±0.27	9.93±0.27	5.16±0.04	22.28±0.40	9.83±0.11	42.85±1.05	7.39±0.17	35.47±0.96	68.65±0.70	4.00±0.15
Subpopulation 2 (P3xP5)	137	126.96±1.17	29.64±0.25	20.97±0.16	8.68±0.18	5.23±0.03	21.02±0.28	9.65±0.08	40.98±0.72	7.53±0.12	33.48±0.67	64.84±0.40	5.50±0.11
Subpopulation 3 (P4xP5)	48	127.38±1.64	27.97±0.35	19.36±0.21	8.63±0.31	5.00±0.04	22.43±0.42	9.62±0.12	41.94±1.15	6.50±0.18	35.43±1.07	64.86±0.48	5.22±0.20
Subpopulation 4 (P2xP5)	78	124.15±1.34	31.28±0.33	20.42±0.15	10.84±0.24	5.11±0.05	21.71±0.32	10.09±0.11	47.56±1.11	7.08±0.14	40.48±1.04	64.89±0.38	4.24±0.16
Subpopulation 5 (P3xP6)	34	140.34±2.25	31.39±0.44	21.27±0.33	10.12±0.33	5.01±0.05	23.67±0.41	10.97±0.14	55.15±1.63	10.02±0.31	45.13±1.46	64.05±0.62	5.01±0.28
Subpopulation 6 (P1xP6)	23	134.29±2.22	33.22±0.56	22.46±0.48	10.76±0.39	4.93±0.06	24.06±0.66	10.33±0.18	48.29±1.94	7.68±0.33	40.62±1.79	66.75±0.73	4.39±0.28
Subpopulation 7 (P4xP6)	30	146.55±3.12	32.89±0.44	22.97±0.42	9.91±0.33	5.20±0.06	25.51±0.56	10.90±0.16	58.96±1.81	9.70±0.24	49.27±1.67	63.48±0.60	4.28±0.27
Subpopulation 8 (P2xP6)	57	128.49±1.58	32.57±0.42	21.53±0.32	11.04±0.28	4.98±0.04	22.85±0.39	10.39±0.13	49.02±1.33	8.52±0.18	40.49±1.21	66.29±0.46	3.86±0.20

§ Standard errors are attached.

CL: Cob length, ED: Ear diameter, CD: Cob diameter, KD: Kernel depth, NK: Number of kernels/row, KT: Kernel thickness, NR: Number of rows/ear, EW: Ear weight, CW: Cob weight, TOTALKW: Total kernel weight of ear.

Table 2.6 Phenotypic correlations among pericarp thickness, ear and agronomic traits measured on MAS population in 2008.

	UG	LG	UA	LA	CWN	CL	ED	CD	KD	NK	KT	NR	EW	CW	TOTAL KW	ANT
LG	0.87**															
UA	0.91**	0.84**														
LA	0.84**	0.86**	0.95**													
CWN	0.94**	0.80**	0.95**	0.86**												
CL	-0.06	-0.16**	-0.10*	-0.15**	-0.03											
ED	-0.11*	-0.13**	-0.11*	-0.09	-0.13**	0.18**										
CD	0.02	-0.01	0.03	0.04	0.03	0.14**	0.71**									
KD	-0.17**	-0.17**	-0.18**	-0.16**	-0.21**	0.11*	0.73**	0.04								
NK	-0.16**	-0.30**	-0.18**	-0.26**	-0.11*	0.75**	0.29**	0.12*	0.29**							
KT	0.18**	0.29**	0.17**	0.23**	0.15**	-0.02	-0.16**	0.04	-0.26**	-0.45**						
NR	-0.28**	-0.33**	-0.30**	-0.30**	-0.29**	0.09	0.54**	0.42**	0.38**	0.31**	-0.31**					
EW	-0.22**	-0.35**	-0.24**	-0.30**	-0.20**	0.60**	0.54**	0.33**	0.44**	0.67**	-0.27**	0.50**				
CW	0.04	-0.04	0.06	0.04	0.08	0.47**	0.46**	0.48**	0.20**	0.30**	0.08	0.29**	0.60**			
TOTAL KW	-0.24**	-0.38**	-0.27**	-0.33**	-0.24**	0.58**	0.50**	0.28**	0.45**	0.68**	-0.32**	0.50**	0.99**	0.48**		
ANT	0.09*	0.14**	0.08	0.12*	0.07	-0.11*	0.02	0.05	-0.03	-0.21**	0.17**	-0.10*	-0.17**	0.07	-0.20**	
TILLERS	-0.02	-0.02	-0.04	-0.06	-0.04	-0.04	-0.08	0.01	-0.12**	0.00	-0.05	0.09	0.01	-0.03	0.01	-0.02

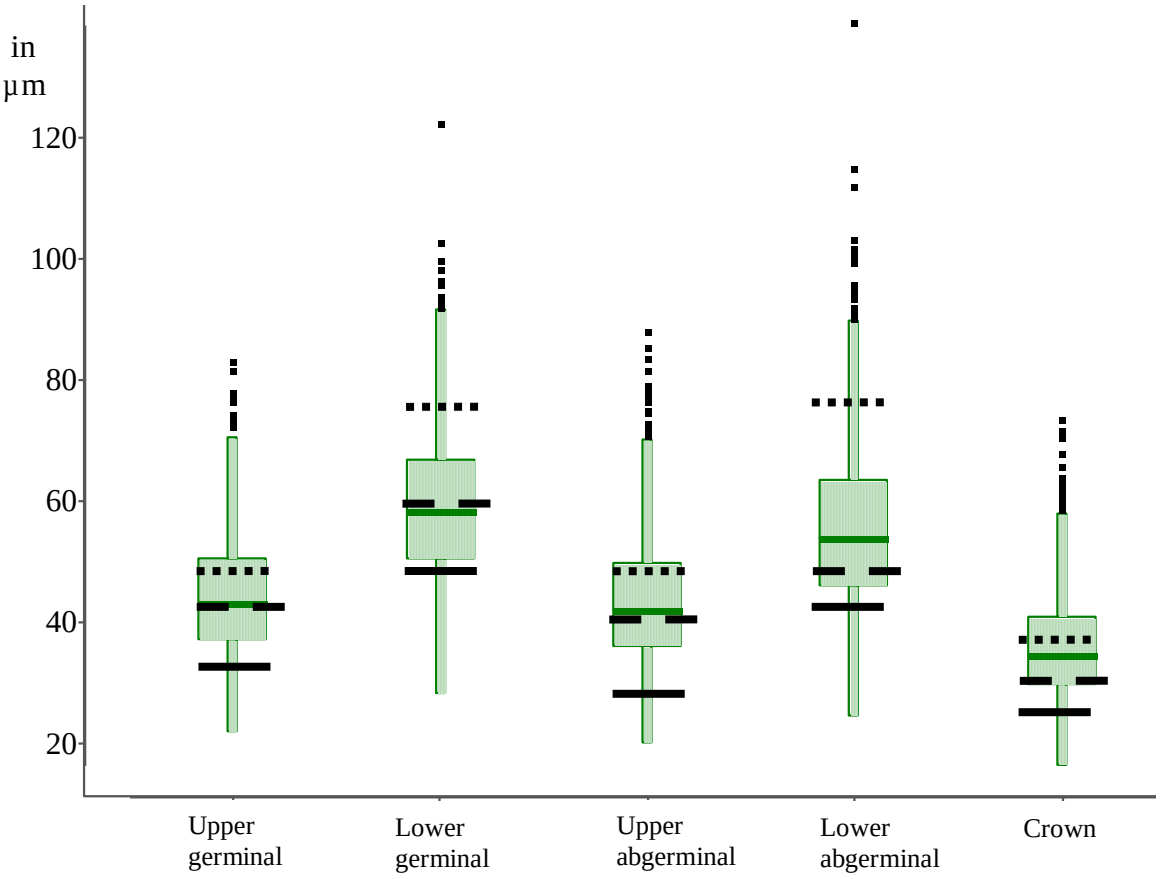
*, ** Phenotypic correlation was significant at the 0.05 and 0.01 probability level, respectively.
ANT: Days to anthesis, TILLERS: Severity of tillers, UG: Upper germinal, LG: Lower germinal, UA: Upper abgerminal, LA: Lower abgerminal, CWN: Crown
CL: Cob length, ED: Ear diameter, CD: Cob diameter, KD: Kernel depth, NK: Number of kernels/row, KT: Kernel thickness, NR: Number of rows/ear,
EW: Ear weight, CW: Cob weight, TOTALKW: Total kernel weight of ear.

Table 2.7 Parameters associated with the three principal components (PC) on pericarp thickness and ear traits and their loadings from MAS population in 2008.

Parameter	Pericarp PC1		
Eigenvalue (λ)	4.43		
% of total variation	88.6		
Pericarp thickness trait loadings			
Upper germinal (UG)	0.45[$]		
Lower germinal (LG)	0.43		
Upper abgerminal (UA)	0.46		
Lower abgerminal (LA)	0.44		
Crown (CWN)	0.45		
Parameter	Ear PC1	Ear PC2	Ear PC3
Eigenvalue (λ)	3.68	1.42	1.21
% of total variation	46.0	17.7	15.1
Ear trait loadings			
Cob length (CL)	**0.37**	**-0.30**	**0.47**
Ear diameter (ED)	**0.39**	**0.35**	-0.21
Cob diameter (CD)	**0.31**	**0.53**	-0.08
Number of kernels/row (NK)	**0.39**	**-0.47**	0.06
Kernel thickness (KT)	-0.16	**0.46**	**0.61**
Number of rows/ear (NR)	**0.33**	0.14	**-0.47**
Ear weight (EW)	**0.45**	-0.12	0.08
Cob weight (CW)	**0.36**	0.21	**0.36**

The bold-faced numbers indicate PC loadings larger than 0.30 and smaller than -0.30 and they were regarded as substantial.

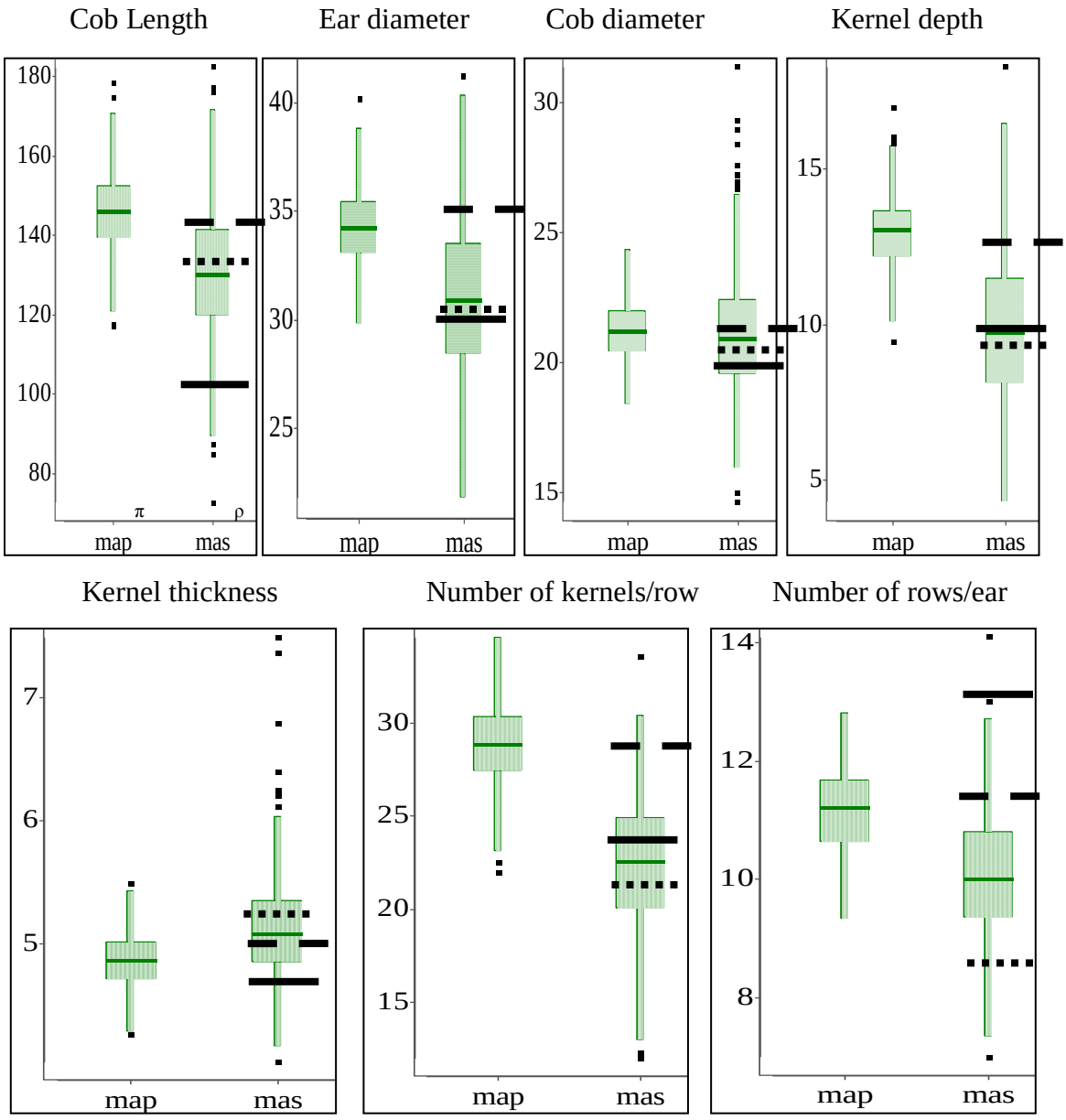
Figure 2.2 Boxplots of MAS populations for pericarp thickness traits measured in 2008 along with the parental and grandparental means.



Means (in µm)					
BH20	54.86	81.80	56.74	81.69	42.02
BH30	35.89	54.14	32.81	42.85	28.17
P1	44.65	69.05	40.10	57.50	30.70
P2	39.05	63.39	40.43	55.96	29.03
P3	56.35	67.85	49.75	66.75	40.20
P4	52.75	69.85	46.15	57.95	40.45
P5	35.27	49.73	35.31	42.08	29.72
P6	38.63	48.90	37.88	46.05	31.58
Means of Parents	44.45	61.46	41.60	54.38	33.61

Mean of BH20 — Mean of BH30 — Mean of parental lines

Figure 2.3 Boxplots of MAS populations for ear traits measured in 2008 along with the boxplots of mapping populations for corresponding ear traits measure in four environments from 2004 to 2006.



π: mapping population measured from 2004 to 2006.
ρ: MAS population measured in 2008.
Mean of BH20. Mean of BH30. Mean of parental lines.

Figure 2.3 Continued.

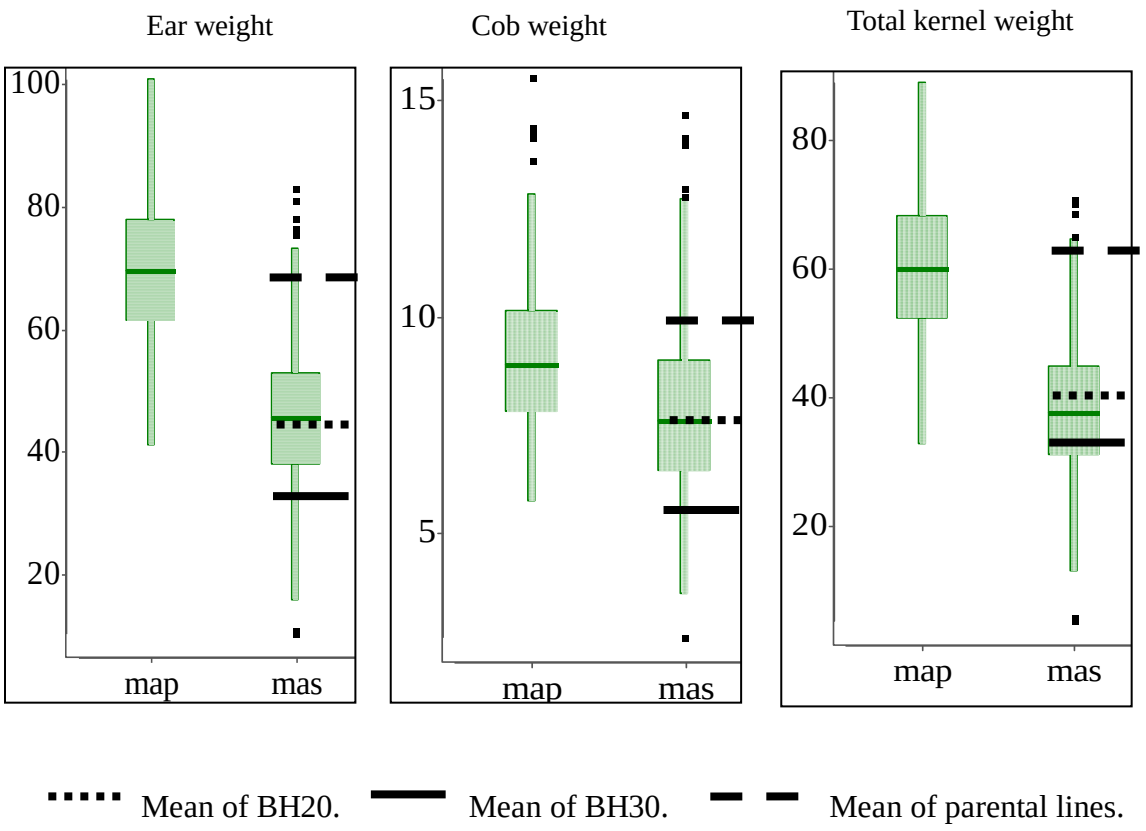


Table 2.8 PC scores of grandparents, parental lines and MAS population including each subpopulstion for pericarp and ear trait PCs.

Source	Entries	Pericarp PC	Ear PC1	Ear PC2	Ear PC3
BH20	5	3.00±0.15§	-0.71±0.28	0.03±0.27	0.90±0.22
BH30	5	-1.78±0.12	0.09±0.83	-1.72±0.24	-3.26±0.37
P1	2	0.06±0.32	3.35±0.45	-1.05±0.30	0.34±0.49
P2	2	-0.51±0.80	3.42±0.31	0.84±0.35	-0.24±0.19
P3	2	1.72±0.22	1.72±0.23	-1.18±0.16	-0.04±0.41
P4	2	1.22±0.09	3.28±0.35	-1.34±0.32	0.62±0.19
P5	4	-1.73±0.32	-0.67±0.38	-0.27±0.24	-0.87±0.26
P6	4	-1.32±0.04	1.88±0.20	-0.18±0.45	-0.44±0.25
MAS population	493				
Subpopulation 1 (P1xP5)	86	0.53±0.16	-0.26±0.20	0.03±0.14	0.26±0.09
Subpopulation 2 (P3xP5)	137	0.71±0.13	-0.63±0.10	0.22±0.07	0.19±0.07
Subpopulation 3 (P4xP5)	48	0.88±0.19	-0.93±0.13	-0.71±0.11	0.09±0.10
Subpopulation 4 (P2xP5)	78	-0.50±0.17	-0.34±0.14	-0.07±0.10	-0.24±0.08
Subpopulation 5 (P3xP6)	34	-1.86±0.18	1.03±0.18	-0.01±0.13	0.24±0.14
Subpopulation 6 (P1xP6)	23	-1.52±0.23	0.85±0.22	0.01±0.18	-0.33±0.14
Subpopulation 7 (P4xP6)	30	-0.97±0.17	1.60±0.18	0.31±0.16	0.45±0.18
Subpopulation 8 (P2xP6)	57	-0.24±0.19	0.47±0.16	0.14±0.11	-0.26±0.10

§ Standard errors are attached.

Table 2.9 Parameters associated with single factor analysis result on pericarp thickness traits in overall MAS population.

Markers	Traits	Effect	Estimate[ε]	Standard error	tValue	Probability
M01	CWN	additive	4.00	1.14	3.52	0.0005
M01	LA	additive	7.51	1.84	4.09	0.0001
M01	LG	additive	4.49	1.63	2.76	0.0061
M01	PC[a]	additive	10.44	2.80	3.73	0.0002
M01	UA	additive	6.02	1.38	4.37	<0.0001
M01	UG	additive	3.47	1.24	2.80	0.0054
M02	CWN	additive	3.71	1.06	3.49	0.0005
M02	LA	additive	4.93	1.70	2.89	0.0040
M02	LG	additive	3.91	1.43	2.74	0.0065
M02	PC	additive	8.18	2.56	3.19	0.0016
M02	UA	additive	4.11	1.28	3.20	0.0015
M02	UG	additive	3.38	1.15	2.94	0.0035
M04	CWN	additive	6.03	1.07	5.64	<0.0001
M04	LA	additive	8.61	1.80	4.79	<0.0001
M04	LG	additive	7.42	1.54	4.83	<0.0001
M04	PC	additive	14.58	2.66	5.47	<0.0001
M04	UA	additive	7.32	1.32	5.55	<0.0001
M04	UG	additive	6.34	1.16	5.46	<0.0001
M05	CWN	additive	2.88	1.03	2.80	0.0055
M05	UG	additive	2.30	1.12	2.05	0.0415
M07	LG	additive	3.79	1.46	2.60	0.0097
M07	UG	additive	2.53	1.14	2.23	0.0266
M09	LG	additive	-3.08	1.38	-2.22	0.0266
M11	CWN	additive	3.49	1.07	3.28	0.0011
M11	LA	additive	4.57	1.73	2.64	0.0087
M11	LG	additive	5.06	1.48	3.41	0.0007
M11	PC	additive	8.46	2.60	3.25	0.0012
M11	UA	additive	3.59	1.30	2.76	0.0061
M11	UG	additive	4.05	1.13	3.58	0.0004
M12	LG	additive	3.51	1.38	2.54	0.0115
M14	CWN	additive	-2.75	1.09	-2.51	0.0125
M14	LA	additive	-4.20	1.77	-2.37	0.0183
M14	PC	additive	-5.75	2.72	-2.12	0.0351
M14	UA	additive	-3.24	1.33	-2.44	0.0153
M16	CWN	additive	-4.15	1.16	-3.58	0.0004
M16	LA	additive	-9.09	1.76	-5.17	<0.0001
M16	LG	additive	-6.45	1.61	-4.01	0.0001
M16	PC	additive	-12.41	2.76	-4.50	<0.0001
M16	UA	additive	-6.68	1.38	-4.86	<0.0001

Table 2.9 Continued.

M16	UG	additive	-3.93	1.25	-3.14	0.0019
M02	CWN	dominance	-4.48	2.22	-2.02	0.0445
M02	LA	dominance	-8.39	3.57	-2.35	0.0193
M02	LG	dominance	-7.79	2.99	-2.61	0.0096
M02	PC	dominance	-12.39	5.37	-2.31	0.0216
M02	UA	dominance	-5.78	2.69	-2.15	0.0320
M08	CWN	dominance	-5.12	1.75	-2.92	0.0037
M08	UA	dominance	-4.83	2.16	-2.23	0.0263
M08	UG	dominance	-4.22	1.90	-2.22	0.0271
M09	LA	dominance	-6.53	2.49	-2.62	0.0090
M09	LG	dominance	-4.75	2.15	-2.21	0.0274
M09	PC	dominance	-7.96	3.78	-2.11	0.0355
M09	UA	dominance	-4.28	1.88	-2.28	0.0233
M16	LA	dominance	7.16	2.90	2.47	0.0142

a PC was calculated based on pericarp PC from MAS population in 2008.

ε The additive effect of each marker was calculated as (mean of the BH20 genotypic class – mean of BH30 genotypic class). Therefore, positive values indicate that BH30 carries the allele for decreasing peicarp thickness, and negative values indicate that BH20 contributes the alleles for decreasing pericarp thickness.

The dominance effect of each marker was calculated as (mean of homozygous for BH20 and BH30-mean of heterozygote). Therefore, positive values indicate that heterozygote has the effect of decreasing pericarp thickness, and negative values indicate that homozygous of BH20 and BH30 have the effect of decreasing pericarp thickness.

Table 2.10 Parameters associated with single factor analysis result on pericarp thickness traits in each subpopulation.

Markers	Population	Traits	Effect [ε]	Estimate	Standard error	tValue	Probability
M01	2	UG	additive	3.83	1.86	2.06	0.0419
M01	2	UA	additive	5.44	2.00	2.72	0.0075
M01	2	LA	additive	7.40	2.93	2.53	0.0129
M01	2	CWN	additive	3.84	1.66	2.32	0.0223
M01	2	PC[a]	additive	10.28	4.19	2.46	0.0156
M01	4	UA	additive	9.42	3.24	2.90	0.0052
M01	4	LA	additive	11.35	4.15	2.74	0.0082
M01	4	CWN	additive	5.07	2.42	2.09	0.0409
M04	1	UG	additive	9.24	2.17	4.25	<0.0001
M04	1	LG	additive	11.71	2.69	4.34	<0.0001
M04	1	UA	additive	10.37	2.29	4.53	<0.0001
M04	1	LA	additive	13.36	3.31	4.03	0.0001
M04	1	CWN	additive	8.48	1.96	4.32	<0.0001
M04	1	PC	additive	21.70	4.73	4.59	<0.0001
M04	2	LG	additive	6.99	3.50	2.00	0.0484
M04	2	UA	additive	6.25	2.93	2.13	0.0354
M04	2	LA	additive	8.48	4.21	2.02	0.0463
M04	2	PC	additive	12.52	6.10	2.05	0.0425
M04	3	UG	additive	7.61	3.44	2.21	0.0337
M04	3	UA	additive	14.27	4.26	3.35	0.0019
M04	3	LA	additive	19.12	6.03	3.17	0.0031
M04	3	CWN	additive	8.18	3.28	2.49	0.0175
M04	3	PC	additive	23.51	7.98	2.95	0.0057
M04	5	UG	additive	6.01	2.65	2.26	0.0326
M04	5	UA	additive	9.09	3.07	2.96	0.0067
M04	5	LA	additive	11.87	4.74	2.50	0.0192
M04	5	CWN	additive	6.61	2.11	3.14	0.0043
M04	5	PC	additive	16.97	6.37	2.66	0.0133
M04	6	UG	additive	10.73	3.76	2.85	0.0146
M04	6	LG	additive	11.49	4.75	2.42	0.0324
M04	6	UA	additive	10.77	3.52	3.06	0.0099
M04	6	LA	additive	15.18	4.48	3.39	0.0054
M04	6	PC	additive	22.65	7.47	3.03	0.0105
M05	5	UG	additive	-10.58	3.41	-3.10	0.0068
M05	5	LG	additive	-15.91	4.74	-3.36	0.0040
M05	5	UA	additive	-11.28	4.19	-2.69	0.0160

Table 2.10 Continued.

M05	5	LA	additive	-18.90	5.90	-3.21	0.0055
M05	5	CWN	additive	-6.35	2.97	-2.14	0.0483
M05	5	PC	additive	-25.78	8.24	-3.13	0.0065
M07	6	LG	additive	15.15	6.29	2.41	0.0294
M07	7	LG	additive	8.36	3.95	2.12	0.0469
M08	3	UG	additive	-8.73	3.06	-2.86	0.0070
M08	3	UA	additive	-12.82	4.04	-3.18	0.0030
M08	3	LA	additive	-14.08	5.28	-2.67	0.0113
M08	3	CWN	additive	-9.91	2.91	-3.40	0.0016
M08	3	PC	additive	-21.69	7.48	-2.90	0.0062
M08	4	UG	additive	-12.17	3.07	-3.97	0.0002
M08	4	LG	additive	-13.70	3.75	-3.66	0.0005
M08	4	UA	additive	-19.53	3.93	-4.97	<0.0001
M08	4	LA	additive	-24.69	5.09	-4.85	<0.0001
M08	4	CWN	additive	-11.87	2.96	-4.01	0.0002
M08	4	PC	additive	-33.57	7.19	-4.67	<0.0001
M09	2	LG	additive	5.38	2.64	2.04	0.0432
M10	5	UG	additive	-8.02	1.64	-4.89	<0.0001
M10	5	UA	additive	-8.33	2.84	-2.94	0.0082
M10	5	LA	additive	-12.22	4.48	-2.73	0.0129
M10	5	CWN	additive	-5.25	1.80	-2.91	0.0086
M10	5	PC	additive	-16.54	5.33	-3.10	0.0056
M11	7	CWN	additive	13.17	5.65	2.33	0.0281
M12	1	LG	additive	7.91	3.25	2.44	0.0172
M12	1	LA	additive	9.76	3.83	2.55	0.0129
M12	1	PC	additive	13.05	5.76	2.26	0.0265
M12	5	UA	additive	-7.46	3.14	-2.38	0.0253
M12	5	CWN	additive	-5.55	2.22	-2.50	0.0193
M13	2	CWN	additive	4.39	1.81	2.43	0.0166
M13	4	LG	additive	7.68	3.18	2.42	0.0190
M14	1	UA	additive	-5.70	2.82	-2.02	0.0481
M14	1	CWN	additive	-4.99	2.27	-2.20	0.0322
M14	2	CWN	additive	-3.88	1.85	-2.09	0.0398
M14	4	LG	additive	-7.12	2.90	-2.45	0.0180
M14	4	LA	additive	-7.06	3.47	-2.03	0.0479
M14	4	PC	additive	-10.91	5.19	-2.10	0.0408
M15	8	LG	additive	13.27	6.40	2.07	0.0440
M15	8	UA	additive	15.78	6.37	2.48	0.0171
M15	8	LA	additive	19.43	7.39	2.63	0.0117

Table 2.10 Continued.

M15	8	CWN	additive	11.72	5.58	2.10	0.0416
M15	8	PC	additive	28.56	12.27	2.33	0.0246
M16	1	UG	additive	10.23	4.04	2.53	0.0155
M16	1	CWN	additive	8.80	3.58	2.46	0.0186
M16	2	UA	additive	-7.51	2.67	-2.81	0.0061
M16	2	LA	additive	-12.02	3.50	-3.44	0.0009
M16	2	PC	additive	-12.77	5.39	-2.37	0.0200
M16	3	UG	additive	-8.96	4.15	-2.16	0.0426
M16	3	UA	additive	-11.95	4.87	-2.46	0.0229
M16	3	LA	additive	-14.88	6.72	-2.22	0.0379
M16	3	CWN	additive	-8.67	3.69	-2.35	0.0286
M01	4	UG	dominance	-13.63	4.66	-2.93	0.0049
M01	4	LG	dominance	-21.44	5.53	-3.88	0.0003
M01	4	UA	dominance	-15.00	5.83	-2.57	0.0128
M01	4	LA	dominance	-16.66	7.46	-2.23	0.0295
M01	4	CWN	dominance	-12.74	4.36	-2.92	0.0050
M01	4	PC	dominance	-32.41	10.79	-3.00	0.0040
M01	7	LG	dominance	13.92	4.85	2.87	0.0092
M02	8	UA	dominance	-27.26	10.73	-2.54	0.0158
M02	8	CWN	dominance	-21.47	9.17	-2.34	0.0252
M04	1	LA	dominance	-12.31	5.70	-2.16	0.0339
M04	1	PC	dominance	-16.70	8.13	-2.05	0.0436
M04	3	UA	dominance	-14.02	6.79	-2.06	0.0465
M04	3	LA	dominance	-22.29	9.62	-2.32	0.0265
M04	6	UG	dominance	13.97	5.75	2.43	0.0318
M04	6	LG	dominance	20.54	7.26	2.83	0.0152
M04	6	UA	dominance	13.46	5.38	2.50	0.0278
M04	6	LA	dominance	19.62	6.85	2.86	0.0142
M04	6	PC	dominance	31.62	11.42	2.77	0.0170
M04	8	CWN	dominance	10.87	5.32	2.04	0.0475
M05	2	LA	dominance	-15.68	7.81	-2.01	0.0482
M06	2	UG	dominance	10.49	3.91	2.68	0.0086
M06	2	LG	dominance	12.26	5.13	2.39	0.0188
M06	2	UA	dominance	9.66	4.25	2.27	0.0250
M06	2	CWN	dominance	8.39	3.51	2.39	0.0187
M06	2	PC	dominance	21.06	8.90	2.37	0.0198
M07	4	UG	dominance	-18.59	4.59	-4.05	0.0002
M07	4	LG	dominance	-18.88	5.35	-3.53	0.0009
M07	4	UA	dominance	-26.24	6.23	-4.21	0.0001

Table 2.10 Continued.

M07	4	LA	dominance	-33.24	7.76	-4.28	<0.0001
M07	4	CWN	dominance	-19.15	4.64	-4.13	0.0001
M07	4	PC	dominance	-47.49	10.94	-4.34	<0.0001
M07	8	UG	dominance	13.13	6.12	2.15	0.0371
M07	8	LG	dominance	18.75	6.91	2.71	0.0093
M07	8	UA	dominance	15.29	7.01	2.18	0.0342
M07	8	LA	dominance	18.05	7.98	2.26	0.0284
M07	8	PC	dominance	30.86	13.31	2.32	0.0249
M08	7	UG	dominance	-9.82	4.62	-2.13	0.0443
M10	5	UG	dominance	-12.06	2.60	-4.63	0.0002
M10	5	LG	dominance	-22.28	5.78	-3.86	0.0010
M10	5	UA	dominance	-11.49	4.51	-2.55	0.0192
M10	5	LA	dominance	-19.43	7.11	-2.73	0.0128
M10	5	CWN	dominance	-7.75	2.86	-2.71	0.0135
M10	5	PC	dominance	-29.83	8.47	-3.52	0.0021
M12	2	UG	dominance	-6.56	3.24	-2.03	0.0452
M13	2	LG	dominance	-12.47	5.38	-2.32	0.0223
M14	2	UG	dominance	11.43	5.68	2.01	0.0474
M14	2	LG	dominance	15.99	7.27	2.20	0.0307
M14	2	UA	dominance	15.17	6.27	2.42	0.0179
M14	2	LA	dominance	19.00	9.17	2.07	0.0416
M14	2	CWN	dominance	12.84	5.05	2.54	0.0130
M14	2	PC	dominance	30.41	12.88	2.36	0.0208
M16	4	LG	dominance	12.03	4.83	2.49	0.0172
M16	4	UA	dominance	13.50	4.84	2.79	0.0082
M16	4	LA	dominance	21.04	6.22	3.38	0.0017
M16	4	CWN	dominance	8.41	3.48	2.42	0.0206
M16	4	PC	dominance	25.40	8.52	2.98	0.0050

UG: Upper germinal, LG: Lower germinal, UA: Upper abgerminal, LA: Lower abgerminal, CWN: Crown, PC: Principal component for pericarp thickness traits

a PC was calculated based on pericarp PC from MAS population in 2008.

ε The additive effect of each marker was calculated as (mean of the BH20 genotypic class – mean of BH30 genotypic class). Therefore, positive values indicate that BH30 carries the allele for decreasing peicarp thickness, and negative values indicate that BH20 contributes the alleles for decreasing pericarp thickness.

The dominance effect of each marker was calculated as (mean of homozygous for BH20 and BH30-mean of heterozygote). Therefore, positive values indicate that heterozygote has the effect of decreasing pericarp thickness, and negative values indicate that homozygous of BH20 and BH30 have the effect of decreasing pericarp thickness.

Table 2.11 Parameters associated with single factor analysis result on ear traits in overall MAS population.

	Trait	Effect	Estimate	Standard error	tValue	Probability
M02	CD	additive	0.64	0.30	2.17	0.0309
M02	NR	additive	-0.31	0.14	-2.26	0.0244
M03	KT	additive	-0.13	0.06	-2.26	0.0244
M03	ED	additive	0.98	0.46	2.10	0.0362
M03	NR	additive	0.51	0.15	3.36	0.0008
M04	KT	additive	0.17	0.06	2.82	0.0050
M04	ED	additive	-1.36	0.46	-2.96	0.0033
M05	NK	additive	1.03	0.46	2.25	0.0251
M05	CL	additive	6.88	1.99	3.45	0.0006
M05	CW	additive	0.61	0.24	2.53	0.0119
M06	EW	additive	-4.50	1.75	-2.57	0.0106
M06	NR	additive	-0.45	0.17	-2.72	0.0067
M06	CW	additive	-0.57	0.28	-2.02	0.0438
M07	KT	additive	0.14	0.05	2.85	0.0046
M07	NR	additive	-0.39	0.14	-2.81	0.0051
M07	CW	additive	0.48	0.24	1.97	0.0492
M08	CD	additive	-0.58	0.29	-2.00	0.0465
M08	KT	additive	0.19	0.05	3.63	0.0003
M08	CW	additive	-0.50	0.23	-2.11	0.0353
M09	EW	additive	4.73	1.41	3.36	0.0008
M09	NK	additive	1.40	0.43	3.29	0.0011
M09	CL	additive	8.73	1.92	4.55	<0.0001
M09	CW	additive	1.12	0.23	4.90	<0.0001
M11	EW	additive	-5.10	1.50	-3.40	0.0007
M11	NK	additive	-1.02	0.45	-2.26	0.0244
M11	CL	additive	-8.19	2.08	-3.95	<0.0001
M12	NR	additive	-0.52	0.13	-3.98	<0.0001

CL: Cob length, ED: Ear diameter, CD: Cob diameter, NK: Number of kernels/row, KT: Kernel thickness, NR: Number of rows/ear, EW: Ear weight, CW: Cob weight

ε The additive effect of each marker was calculated as (mean of the BH20 genotypic class – mean of BH30 genotypic class). Therefore, positive values indicate that BH20 carries the allele for increasing ear traits, and negative values indicate that BH30 contribute the alleles for increasing ear traits.

The dominance effect of each marker was calculated as (mean of homozygous for BH20 and BH30-mean of heterozygote). Therefore, positive values indicate that homozygous genotypes have the effect of increasing ear traits, and negative values indicate that heterozygotes for BH20 and BH30 have the effect of increasing ear traits.

Table 2.12 List of pericarp thickness and ear traits significant for the markers in MAS and mapping populations measured in 2008 and in 2004-2006 respectively.

Markers	QTL (Bin)	Pericarp thickness traits		Ear traits	Pericarp thickness traits significant in mapping population
		Additive	Dominance		
M01	QTL1 (2.06)	UG, LG, UA, LA, C, PC	-	-	UG (8.9)†, LG (8.4), UA (12.4), LA (7.7), CWN (7.8), PC (10.6)
M02		UG, LG, UA, LA, C, PC	LG, UA, LA, CWN, PC	CD, NR	
M03	QTL2 (3.00)	-	-	KT, ED, NR	UG(8.9), LG (8.1), UA (13.4), LA (12.4), CWN (6.5), PC (8.4)
M04		UG, LG, UA, LA, C, PC	-	KT, ED	
M05	QTL3 (4.01)	UG, CWN	-	NK, CL, CW	UG (7.5), PC (6.9)
M06		-	-	EW, NR, CW	
M07	QTL4 (4.07)	UG, LG	-	KT, NR, CW	UG (5.6), LG (10.7), PC (6.3)
M08		-	UG, UA, CWN	CD, KT, CW	
M09	QTL5 (6.00)	LG	LG, UA, LA, PC	EW, NK, CL, CW	UG (7.0)
M10	QTL6 (8.05)	-	-	-	
M11		UG, LG, UA, LA, C, PC	-	EW, NK, CL	LG (7.4)
M12	QTL7 (9.03)	LG	-	NR	UA (7.4), LA (5.6), CWN (11.1), PC (5.8)
M16	QTL8 (1.10)	UG, LG, UA, LA, C, PC	LA	-	UA (9.7), LA (10.8), CWN (10.0), PC (8.5)
M13		-	-	-	
M14	QTL9 (6.05)	UA, LA, CWN, PC	-	-	UG (10.0), PC (6.0)
M15		-	-	-	

† Proportion of phenotypic variation accounted for QTL in mapping population.
UG: Upper germinal, LG: Lower germinal, UA: Upper abgerminal, LA: Lower abgerminal, CWN: Crown, PC: Principal component for pericarp thickness traits
CL: Cob length, ED: Ear diameter, CD: Cob diameter, KD: Kernel depth, NK: Number of kernels/row, KT: Kernel thickness, NR: Number of rows/ear, EW: Ear weight, CW: Cob weight.

Table 2.13 List of number of pericarp thickness traits with additive and dominance effects significant for markers within subpopulations in MAS population.

Bin	QTL	Marker	Sub-population1	Sub-population2	Sub-population3	Sub-population4	Sub-population5	Sub-population6	Sub-population7	Sub-population8	Total
2.06	QTL1	M01	-	5	-	3(6)	-	-	(1)	-	8
		M02	-	-	-	-	-	-	-	(2)	0
3.00	QTL2	M03	-	-	-	-	-	-	-	-	0
		M04	6(2)$^{\lambda}$	4	5(2)	-	5	5(5)	-	(1)	25
4.01	QTL3	M05	-	(1)	-	-	6	-	-	-	6
		M06	-	(5)	-	-	-	-	-	-	0
4.07	QTL4	M07	-	-	-	(6)	-	1	1	(5)	2
		M08	-	-	5	6	-	-	(1)	-	11
6.00	QTL5	M09	-	1	-	-	-	-	-	-	1
8.05	QTL6	M10	-	-	-	-	5(6)	-	-	-	5
		M11	-	-	-	-	-	-	1	-	1
9.03	QTL7	M12	3	(1)	-	-	2	-	-	-	5
1.10	QTL8	M13	-	1(1)	-	1	-	-	-	-	2
		M16	2	3	4	(5)	-	-	-	-	9
6.05	QTL9	M14	2	1(6)	-	3	-	-	-	-	6
		M15	-	-	-	-	-	-	-	5	5
Total			13	15	14	13	18	6	2	5	86

λ The number of pericarp thickness traits significant for dominance effects.

Table 2.14 Means of each pericarp and ear traits and corresponding PC scores on selected lines based on different selection methods, PS, MOST and LEAST from MAS population in 2008.

Method	PS	MOST	LEAST
N	24	25	25
Pericarp thickness traits			
Upper germinal (UG)	29.28±0.52 a†	37.81±0.99 b	49.33±1.73 c
Lower germinal (LG)	39.69±1.07 a	52.51±1.68 b	65.87±2.08 c
Upper abgerminal (UA)	27.27±0.57 a	35.94±1.08 b	48.73±1.58 c
Lower abgerminal (LA)	34.08±0.69 a	46.46±1.47 b	62.32±2.46 c
Crown (CWN)	23.05±0.49 a	29.39±0.92 b	40.33±1.27 c
Pericarp PC	-3.31±0.10 a	-1.41±0.21 b	1.15±0.33 c
Ear traits			
Cob length (CL)	136.55±3.07 a	133.45±2.30 a	135.27±3.26 a
Ear diameter (ED)	31.19±0.57 a	32.01±0.63 a	30.47±0.57 a
Cob diameter (CD)	20.78±0.25 a	20.45±0.33 a	20.84±0.44 a
Kernel thickness (KT)	4.98±0.07 a	4.91±0.07 a	5.13±0.08 a
Number of kernels/row (NK)	24.22±0.63 a	24.11±0.52 a	22.77±0.72 a
Number of rows/ear (NR)	10.59±0.22 a	10.35±0.16 ab	9.77±0.20 b
Ear weight (EW)	52.23±2.43 a	51.82±2.36 a	45.74±1.93 a
Cob weight (CW)	8.24±0.48 a	7.44±0.36 a	8.46±0.39 a
Total kernel weight of ear (TOTALKW)	43.99±2.13 ab	44.37±2.19 a	37.28±1.73 b

† Means within a column followed by the same letter are not different at the 0.05 probability level.

Figure 2.4 Boxplots of pericarp thickness traits and corresponding PC scores for selected lines based on PS, MOST and LEAST selection methods from MAS population. ▪▪▪▪▪▪ Mean value of BH20 ——— Mean value of BH30

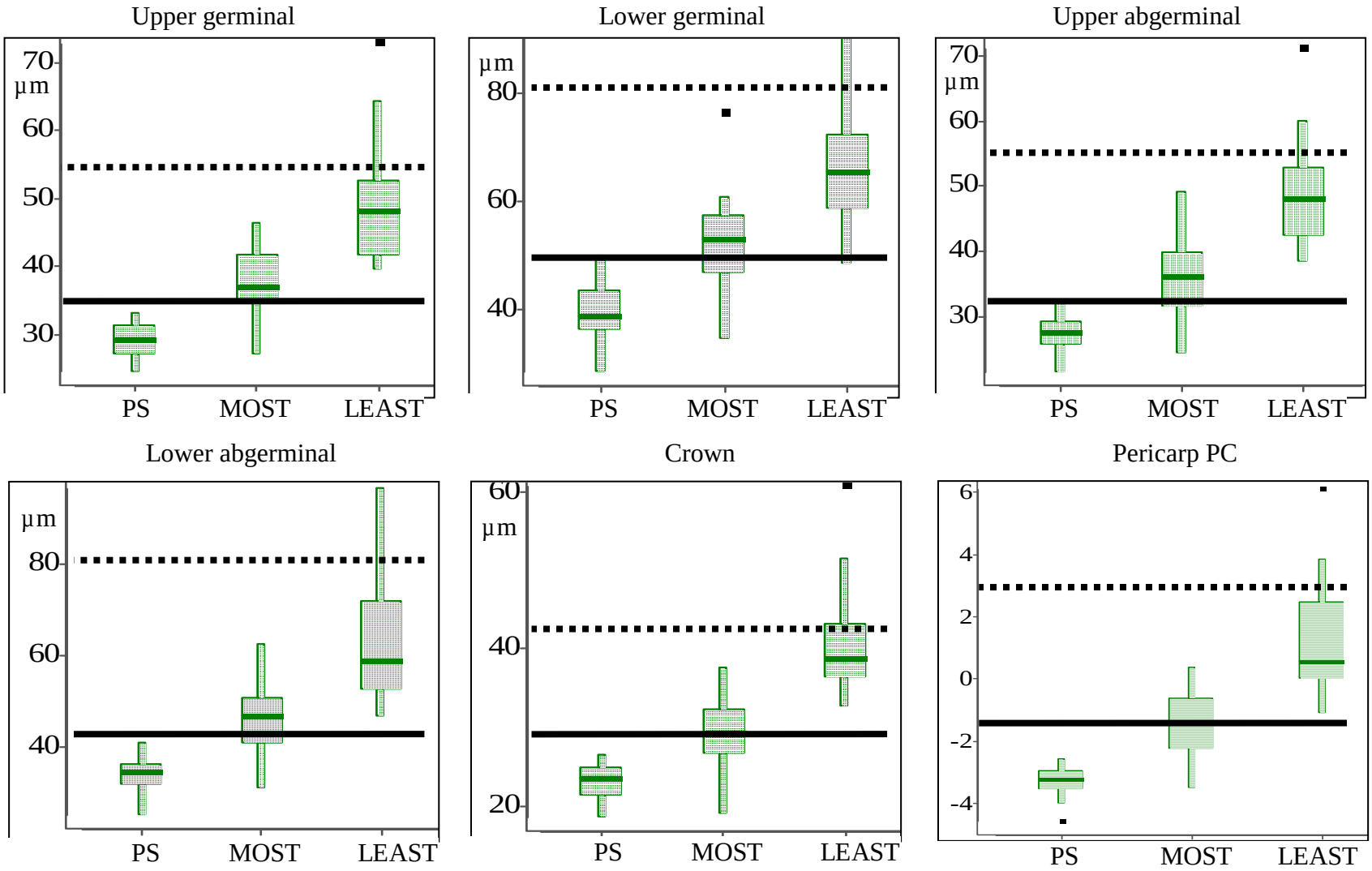


Figure 2.5 Boxplots of pericarp thickness traits and pericarp PC scores for different percentages of favorable QTL alleles in MAS population.

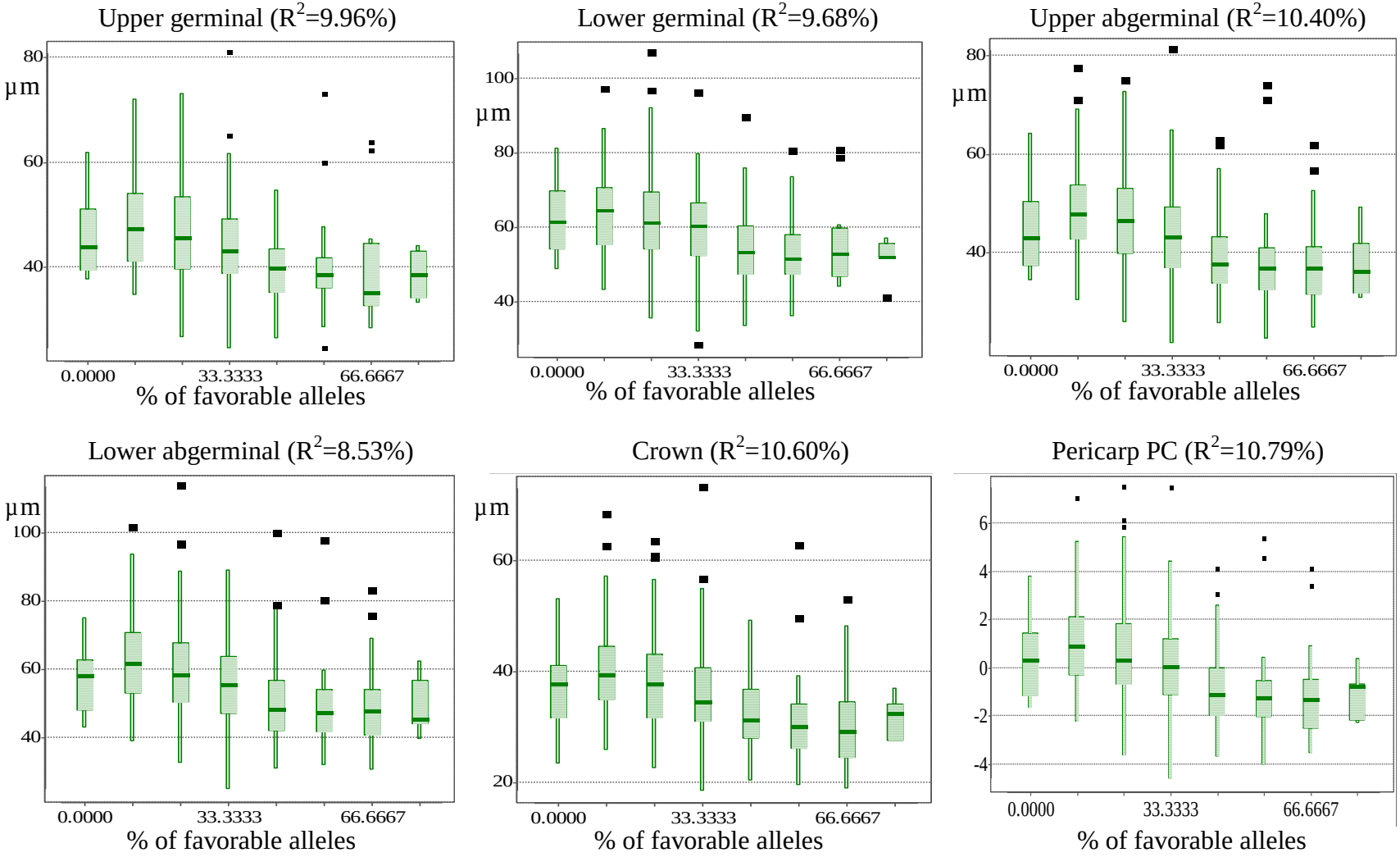


Table 2.15 Pericarp thickness trait values and PC scores of the selected best line based on pericarp thickness phenotypes and the selected best line based on pericarp thickness favorable QTL alleles on each subpopulation measured in 2008.

Best for pericarp thickness phenotypes (sorted by PC scores)							
Source	Sub population	Pericarp PC	Upper germinal	Lower germinal	Upper abgerminal	Lower abgerminal	Crown
127-1	5	-4.58	24.80	29.20	20.80	24.00	19.20
107-4	6	-3.51	31.50	50.75	24.75	32.50	20.50
66-2	1	-3.47	34.60	47.40	27.20	38.80	24.40
132-6	7	-3.25	25.80	39.00	27.80	33.60	25.60
111-5	8	-3.09	39.40	51.00	32.60	39.80	27.20
59-4	3	-3.07	35.60	47.40	29.25	31.75	28.25
27-4	4	-2.98	33.60	48.80	31.40	41.20	21.20
36-4	2	-2.40	32.60	41.60	30.40	37.40	25.40
Average		-3.29					

Best for pericarp thickness favorable QTL alleles (sorted by percentage of favorable alleles)							
Source	Sub population	Pericarp PC	Upper germinal	Lower germinal	Upper abgerminal	Lower abgerminal	Crown
100-6	5	-2.27	37.80	43.80	33.60	47.40	29.40
15-2	1	-1.87	37.60	46.20	37.60	45.80	30.00
106-2	6	-3.50	28.40	36.40	31.40	36.40	25.20
75-5	4	0.36	46.80	57.80	58.80	71.20	40.60
48-1	2	-2.04	33.60	39.00	31.40	34.60	27.40
112-2	8	-1.48	37.20	45.20	34.20	42.20	31.00
132-7	7	-1.48	35.20	45.80	36.00	45.75	28.60
59-2	3	-0.79	46.60	57.60	35.40	44.20	35.00
Average		-1.63					

CHAPTER THREE

COMPARISON BETWEEN PER SE AND TESTCROSS PERFORMANCE FOR PERICARP THICKNESS AND EAR TRAITS FOR SELECTED LINES IN FRESH WAXY CORN

ABSTRACT

Testcross performance (TP) provides important information complementary to per se line performance (LP) in a fresh waxy corn breeding program. We assessed TP of three-way hybrids developed from two groups of selected lines from a MAS population and then crossed with two different hybrid testers BH10xBH20 (BH1020) and BH10xBH30 (BH1030). This was done to: 1) compare TP with the corresponding LP for the selected lines, and 2) compare performance of Group 1 with the most favorable QTL alleles and Group 2 with the fewest favorable QTL alleles, and 3) evaluate performance of pericarp thickness and ear traits between the two different but related testers. Group 1 showed significantly thinner pericarp than Group 2 in testcross evaluation regardless of tester. The three-way hybrids involving tester BH1030 (TP2), which was the thinner pericarp testcross hybrid, showed thinner pericarp than three-way hybrids involving tester BH1020 (TP1). Comparison of the midparent values for pericarp thickness traits showed that both testers, BH1020 and BH1030, had dominance effects on reducing pericarp thickness in three-way hybrids, and the dominance effects of BH1030 were larger than BH1020. There were limited group effects for ear traits, with only CL and KT significantly different. The tester BH1020 showed more positive dominance effects than BH1030 for most of ear traits. There were relatively low correlations between LP and TP for most ear traits, except for CD, KT and NR.

This may imply that dominance plays a substantial role on most ear traits in the three-way hybrids. High correlations between LP and TP for all pericarp thickness traits suggests that indirect improvement in hybrid performance for pericarp traits can be achieved through per se selection.

INTRODUCTION

In maize breeding programs, per se performance as well as testcross performance of lines is important because the performance of a targeted trait may differ between the two. Early generation testing in breeding schemes is used to assess combining ability of lines and to explore the testcross performance of a trait early in the per se inbreeding process. Early generation evaluation of testcrosses can be effective, with some limitations for traits with very low heritability. However, early generation testcross evaluation is generally much more expensive than per se evaluation.

Estimating correlations between per se line performance (LP) and testcross performance (TP) is important for hybrid breeding because when LP and TP show a positive high correlation, simultaneous improvement in the lines and hybrids can be better achieved. Therefore, for traits with high correlation between LP and TP, selection based on LP in early generations can be effective in improving TP, which would be economically advantageous.

The correlations between LP and TP vary considerably for different traits. Traits with small heterotic effects such as grain moisture or ear length showed medium to high correlations, whereas traits with large heterotic effects such as grain yield show low correlations (Hallauer and Miranda, 1988). Many factors such as inbreeding, overdominance, epistasis, and linkage can be

involved in lower correlations for certain traits (Schnell, 1961; Smith, 1986). The effect of heterozygosity level in testcrosses may also reduce the correlation between LP and TP for heterotic traits (Mihaljevic et al., 2005). Correlations between LP and TP for traits with high heritability, and presumably mainly additive gene action, such as grain moisture, kernel weight, protein concentration and plant height, were generally high (Mihaljevic et al., 2005). There is very limited genetic information on pericarp thickness traits. Assessing TP in comparison to LP for fresh waxy corn, which has not been reported on previously, will provide useful information for fresh waxy corn hybrid breeding programs.

Dominant allele effects of the tester in testcross progeny can mask the effects of QTL (Lubberstedt et al., 1997; Melchinger et al., 1998; Austin et al., 2000; Ajmone Marsan et al., 2001) and reduce the correlation between LP and TP (Smith 1986). The consistency of QTL effects estimated in LP varies in different TP genetic backgrounds (Melchinger et al., 1998) and is important in hybrid breeding. Therefore, the per se lines we selected based on QTL information from a previous LP study may perform differently with different testers. The QTL analyses on LP and TP have shown that QTL results are consistent on traits with additive gene action and high correlations between LP and TP, such as grain moisture, kernel weight, protein concentration, and plant height (Mihaljevic et al., 2005; Papst et al., 2004). Because testers and potential hybrid partners of new per se lines are often not set or may change over time, the consistency of QTL for TC performance with different testers is an important consideration in breeding programs.

Choosing testers is critical for testcross performance due to their differences in influencing genetic variance (Hallauer and Miranda, 1988). Commonly, the logical tester in hybrid breeding is a superior inbred from an opposite heterotic group. A study by Hallauer and

Lopez-Perez showed that the ideal tester not only maximizes variance of testcrosses but also maximizes the mean of the targeted trait (1979). In another study, testcross evaluation was performed on a set of RILs, using three different testers (Frascaroli et al., 2009). The testers were the two parents of the RIL population, one a high and one a poor performing line, and an unrelated inbred line. The study showed the tester was associated with differences in testcross performance and QTL detection. The unrelated inbred was more effective in providing useful testcross performance data for the traits with additive effects, such as days to pollen shedding, plant height, kernel moisture and kernel weight, than related inbred lines. In contrast, the poor performing related inbred was the most effective for traits with dominance effects, such as grain yield and number of kernels per plant, among three testers (Frascaroli et al., 2009).

Two commercial hybrids, involving Korean inbreds, BH10 and BH20, and BH20 and BH30, locally produce fresh waxy corn sold in South Korea and in some major cities in U.S. The inbred BH10 is used in waxy hybrids primarily because it has very thin pericarp. This makes it a logical tester in this study for pericarp thickness traits. Hybrids of BH10 x BH20 (BH1020) and BH10 x BH30 (BH1030) were used as testers because the BH10 inbred was not available to make single cross hybrids due to proprietary reasons. The BH10 inbred is not closely related to the lines from the BH20xBH30 mapping population. BH20 has relatively thicker pericarp thickness than BH30. The BH1020 and BH1030 hybrids were used as testers to make three-way hybrids with selected lines derived from BH20xBH30 mapping population. This enabled assessment of tester differences in evaluation of testcross performance for pericarp thickness and ear traits.

In Chapter Two, MAS was used to assess the effect of QTL on pericarp and ear traits in a new generation. The main purpose of that study was to assess the improvement of pericarp traits

by pyramiding favorable QTL alleles while maintaining other valuable phenotypic traits. This involved eight biparental cross derived populations created from families selected from the BH20 x BH30 mapping population. We were able to validate pericarp QTL effects and also to select promising lines for further breeding efforts. We confirmed that pericarp thickness in different kernel regions were highly correlated, and all showed high heritability.

Pericarp of a maize kernel develops from the ovary wall which is maternal tissue. Thickness of pericarp shows differences at different growth stages of kernel. An earlier study reported that the thickness of the ovary wall measured from two sweet corn inbreds and their hybrids showed increase of thickness until ten days for hybrids and fifteen days for inbreds, respectively, and then both decreased in thickness (Haddad, 1931). The number of cell layers in the ovary wall remained the same for both the inbred and hybrid lines during all developmental stages. Because fertilization of maize kernels occurs from the bottom to tip of ears, there may be differences in rate of pericarp development in different part of ears. Based on this consideration, pericarp thickness of top, middle and bottom part of ear was measured in every TC population family in order to get a better understanding of the effect of kernel development on pericarp thickness traits.

Lines from the MAS population based on QTL genotypes were testcrossed with waxy corn F_1 hybrids to (1) compare testcross performance in three-way hybrids with per se performance, (2) evaluate the performance of pericarp thickness and ear traits in testcrosses different but related testers, (3) compare the performance between two groups of lines, one with the most favorable QTL alleles and the other with the fewest, and (4) to assess if there is variation in pericarp thickness on different ear positions of the three-way hybrids.

MATERIALS AND METHODS

Plant Materials

Genotypic information was collected on most families from the overall MAS population prior to planting in spring 2008 for phenotypic evaluation of the MAS study reported in Chapter Two. This genotypic information was used to select two groups of lines. Lines with the most favorable alleles for pericarp thickness QTL comprised Group 1, and lines with the fewest favorable alleles were put in Group 2 (Table 3.2). This selection was based strictly on genotypic information from these lines, and the selection was performed prior to the phenotypic data collection for MAS population in fall and winter 2008-2009. All families were scored by summing marker scores after converting the homozygous favorable allele to 1, the homozygous unfavorable allele to -1, and the heterozygote to 0. Seventeen and thirteen lines were selected for Group 1 and Group 2, respectively.

The Korean inbred lines BH20 and BH30 were grandparents of the MAS population, and with BH10, they are used in the commercial fresh waxy corn hybrids BH10xBH20 (BH1020) and BH20XBH30 (BH1030), due to their good combining ability for agronomic performance and high taste quality. Since the BH10 inbred was not available to make the testcrosses on the University of Illinois South Farms, the hybrids BH1020 and BH1030 were used as testers in this study. This approach enabled use of the BH10 line for testcrossing. The use of two hybrids involving BH20 and BH30 provided a balanced pair of testers avoiding a bias towards just BH20 or BH30 genetic background in the three-way hybrids.

Even though the testers each share one parental inbred with the selected groups, the testcrosses should have enough heterozygosity to be useful for estimating testcross performance.

Testcrossing BH1020 and BH1030 with the selected group of lines resulted in the creation of three-way hybrids. The testers have approximately 50% BH10, accompanied by either 50% BH20 or 50% BH30. Since the selected lines came from BH20xBH30 $F_{2:3}$ MAS population, we could assume that the selected lines have approximately 50% BH20 and 50% BH30. Therefore overall testcross hybrids would have approximately 25% BH10, 50% BH20, and 25% BH30 when crossed to BH1020 and approximately 25% BH10, 50% BH30, and 25% BH20 when crossed to BH1030. BH10 provides a genetically distinct set of alleles for testcrossing. BH20 and BH30 share approximately 50% common genetic background with the lines from BH20xBH30 mapping population.

The (BH20xBH30) F_3 families that were testcrossed had undergone recombination, thus each line on average would be approximately 50% BH20 and 50% BH30, providing useful testcross chromosome segments in contrast to BH10. Conversely, about 50% of each of these lines on average would be identical with BH20 or with BH30, which does not provide a useful contrasting chromosomal segment for the testcross. Therefore, the net average is that each hybrid tester used provided a situation where contrasting chromosome segments in these three-way hybrid are only about 75% of what might be expected in comparison to a single cross hybrid using a genetically distinct inbred tester such as BH10 (Figure 3.1).

Two to three plants of thirty selected lines from the MAS experiment in 2008 were cross-pollinated to the two hybrid testers at University of Illinois, Urbana Champaign South farm. The same $F_{2:3}$ plants used for making testcrosses with the hybrids were also self-pollinated as part of the MAS experiment. Fifteen randomly selected kernels from each three-way hybrid were planted in each of two replications in Puerto Vallarta, Mexico winter nursery in 2008-2009, along with the hybrid testers BH1020 and BH1030 and inbreds BH20 and BH30.

Phenotypic Evaluation

In the testcross experiment, five to seven plants per row were hand self-pollinated, and harvested at maturity for phenotypic data collection. Ear traits were measured on five randomly selected ears per row. Traits measured were cob length (CL), ear diameter (ED), cob diameter (CD), kernel thickness (KT), number of kernels per row (NK), number of rows per ear (NR), ear weight (EW), and cob weight (CW). Kernel depth (KD) and kernel weight (TOTALKW) were calculated from the raw ear trait data set (Table 3.1a). Pericarp thickness traits were measured on five regions (UG, LG, UA, LA and CWN) of kernel for five random kernels from three different ear positions (top, middle and bottom) (Table 3.1a). After assessing the differences in pericarp thickness among different ear position, the data collected on different positions of ear were combined for each line for further analyses.

Statistical Analysis

Analyses of variance (ANOVA) were performed on field data using PROC GLM command in the SAS version 9.1 (SAS Institute Inc., Cary, NC) based on the model: $y_{ijklmn} = \mu + \gamma_i + \alpha_{j(i)} + \beta_{k(i)} + \alpha\beta_{jk(i)} + \varepsilon_{ijkl}, + (\rho_{(ijl)m}) + \varphi_{(ijlm)n}$, where y_{ijklmn} represents the phenotypic pericarp thickness in a region of a single kernel, γ_i the effect of *i*th replication, $\alpha_{j(i)}$ the effect of *j*th tester in the *i*th replication, $\beta_{k(i)}$ the effect of *k*th group in *i*th replication, $\alpha\beta_{jk(i)}$ the interaction between tester and group within replication, and ε_{ijkl} represents residual error. Since values were taken from five random kernels from three different positions of an ear per family per replication for pericarp thickness traits, the effects of the positions were included in the model as $\rho_{(ijl)m}$ for pericarp thickness traits. Also values were taken from five random kernels for pericarp thickness

traits and five random ears per family per replication for ear traits, kernels and plants were also included in the model as subsample, $\varphi_{(ijlm)n}$ to get greater precision of line means.

Adjusted least square line means and standard errors for the testcross population were calculated using PROC GLM command, and mean comparisons for pericarp thickness of different ear regions were performed. Then the mean testcross performance of each group for pericarp thickness and ear traits were calculated. The per se line performance of each group was derived from the mean values for five pericarp thickness and ten ear traits of selected lines from MAS population grown in 2008.

Although testcross population and per se lines were not grown in the same environment, per se line performance from MAS population and testcross performance were compared by group performance in this chapter. Phenotypic correlations were calculated between the testcross performance (which included TP1 for testcross with BH1020 and TP2 for testcross with BH1030) and per se line performance (LP) and also between TP1 and TP2. The results are reported in this chapter is considered preliminary discovery information useful for further study.

We calculated the midparental values between mean per se line performance and tester BH1020 (MP1) and values between mean per se line performance and tester BH1030 (MP2). These values were compared to the corresponding testcross performances, TP1 and TP2, to estimate possible dominance effect of the testers. The dominance effects of the testers were compared with simple t tests.

Table 3.1a List of phenotypic traits measured on testcross population.

Trait	Abbreviation	How measured/calculated
Pericarp thickness traits		
Upper germinal	UG	Thickness of upper germinal region of pericarp in µm
Lower germinal	LG	Thickness of lower germinal region of pericarp in µm
Upper abgerminal	UA	Thickness of upper abgerminal region of pericarp in µm
Lower abgerminal	LA	Thickness of lower abgerminal region of pericarp in µm
Crown	CWN	Thickness of crown region of pericarp in µm
Ear inflorescence architecture traits		
Cob length	CL	Length of the cob in mm
Ear diameter	ED	Diameter of the ear before shelled at the middle of the ear in mm
Cob diameter	CD	Diameter of the cob after shelled at the middle of the cob in mm
Kernel depth	KD	Ear diameter - Cob diameter
Number of kernels/ row	NK	Number of kernels of a random row on ear
Number of rows/ ear	NR	Number of rows per ear at the middle of the ear
Kernel thickness	KT	Length of a kernel in mm = Length of ten kernels in cm at the middle of the ear/10
Ear weight	EW	Weight of the ear before shelled in g
Cob weight	CW	Weight of the cob after shelled in g
Total kernel weight	TOTALKW	Ear weight - Cob weight

Table 3.1b List of abbreviations used in Chapter Three.

Abbreviation	Description
TC	Testcross
LP	Line per se performance
TP	Testcross performance
TP1	Three-way hybrids involving tester BH1020
TP2	Three-way hybrids involving tester BH1030
MP1	The midparent value between the BH1020 tester and mean of selected MAS parental lines
MP2	The midparent value between the BH1030 tester and mean of selected MAS parental lines
BH1020	Hybrid tester BH10xBH20
BH1030	Hybrid tester BH10xBH30
Group 1	Lines with the most favorable QTL alleles
Group 2	Lines with the fewest favorable QTL alleles

RESULTS AND DISCUSSION

QTL Information for Selected Lines

Average proportions of favorable alleles, unfavorable alleles, and heterozygotes for nine pericarp thickness QTL loci genotyped for Group 1 were approximately 60%, 11% and 21% respectively, with 8% missing genotypic data (Table 3.2). The MAS population was derived from crosses between sib-mated $F_{2:3}$ lines for P1, P2, P3 and P4, and $F_{3:4}$ lines for P5 and P6, all of which were segregating for some selected QTL, and we were not able to detect new lines with all favorable alleles homozygous for pericarp thickness QTL. Two lines in Group 1 showed 9 and 12 homozygous favorable alleles and 7 and 3 heterozygous favorable alleles respectively, without any homozygous unfavorable alleles for selected QTL loci. Therefore, improved lines with all homozygous favorable alleles for the selected QTL loci can potentially be achieved by fixing the favorable loci of these two lines. In contrast, average proportions of favorable alleles, unfavorable alleles, and heterozygotes for the QTL loci for Group 2 were about 29%, 47% and 18% respectively with 6% missing genotypic data (Table 3.2). The MAS population was created using four parental lines with favorable QTL alleles crossed with two lines with favorable phenotypic traits. Both parental lines in all crosses contributed at least one favorable allele for the QTL to the MAS population. None of the parental selected lines from MAS population contained homozygous unfavorable alleles for all of 16 flanking marker loci. The highest number of homozygous unfavorable alleles was 10 marker loci out of 16 selected markers for one line in Group 2.

Testcross Performance

The pericarp thickness trait ANOVA result in three-way hybrids showed strong effects of tester hybrids, BH1020 and BH1030. Analysis of Group 1 versus Group 2 was significant for all pericarp thickness traits. The different positions on ear were significant for LG, LA and CWN (Table 3.3).

Pericarp thickness of LG and LA regions of kernels from the middle part of the ear were significantly thinner than corresponding kernel regions from bottom part of the ear. For LG region, pericarp thickness for kernels from the top part of the ear were thicker than that of middle part of ear (Table 3.4).

These results for ear position may have occurred because of morphological differences of kernels in middle part of ear in comparison to the top and bottom part of ear, as opposed to differences in rate of pericarp development in different parts of ear. For the middle part of ear, the lower germinal and abgerminal regions of kernels were easily measured because they had longer germinal and abgerminal regions than kernels from top and bottom parts of the ear. Poorly defined lower germinal and abgerminal regions in top and bottom part of ear may have resulted in obtaining thicker values in pericarp due to measuring error.

For the crown region of kernels, pericarp thickness of the bottom part of the ear was thinner than for the middle part. The crown regions of top and bottom parts of the ear were bigger than that of middle part. Therefore, the region measured may actually be comprised of other regions of kernels that technically do not belong to crown regions. Thus thinner pericarp values in the top and bottom section of ear may be due to measuring error in part. Based on this result, it may be important to select well-formed kernels from the middle section to measure

pericarp thickness accurately and consistently for phenotypic breeding efforts. However, the magnitudes for differences among ear positions were relatively small in general.

For the three-way hybrids, Group 1 showed significantly thinner paricarp than Group 2, for both testers. The TC population with BH1030 tester showed thinner pericarp than with BH1020 tester. Group 2 (less favorable alleles) crossed to BH1030 tester showed thinner pericarp than Group 1 crossed to BH1020 tester for LG, UA and LA, showing the large effect of tester on pericarp thickness (Table 3.5). There were no significant interactions between groups and testers (Data not shown).

The BH10 and BH30 inbreds have a relatively thinner pericarp than BH20, and they contributed to thin pericarp for BH1020 and BH2030 commercial hybrids (personal communication, B. Choe). Pericarp thickness measured for the three hybrids BH1020, BH2030 and BH1030 showed that BH1030 hybrid had the thinnest pericarp, with similar thickness to BH30 (Figure 3.2 and Appendix 3.3). Therefore, the BH1030 hybrid tester appears to have a greater effect than BH1020 on narrower pericarp thickness in 3-way hybrids.

For ear traits, CL and KT were significantly different between Groups (Appendix 3.2). Group 2 showed significantly longer CL and thicker KT than Group 1 (Table 3.6). However, group effects were relatively small. Because we created MAS populations based on pericarp thickness while maintaining favorable ear traits, this result may contribute to no negative associations between favorable thin pericarp and ear traits in testcross populations.

Tester effect was more pervasive than group effect for ear traits in that all ear traits showed significance except CL and EW between testers (Appendix 3.2). Tester BH1030 in TC population (TP2) showed larger in ED, CD, KD, NK, NR and TOTALKW than tester BH1020 in TC population (TP1). Tester BH1020 in TC population (TP1) had larger KT and CW than TP2

(Table 3.6). The tester BH1030 includes BH30 parental inbred which had larger number of kernels than the other two parental inbreds. The tester BH1030 was associated with in more kernels but smaller kernels in TC population. In contrast, TP1 with BH1020 tester resulted in relatively larger KT with larger CW due to effects of BH1020 tester (Table 3.6). Therefore, we could find from two different TP that the BH1030 tester was associated with favorable effects on higher overall ear traits with larger kernel numbers while the BH1020 tester was associated with favorable effects on larger kernel thickness and cob weight traits.

Comparison of Line Per Se and Testcross Performance

Observational comparisons were made between the per se performance of the lines selected for testcrossing and the performance of the testcross hybrids. Since the per se and testcross materials were not grown in the same environment, they were confounded by environment and sound statistical comparisons were not possible. However comparison of these materials, with this caveat in mind, is useful as it provides some preliminary information on possible relationships between per se line and testcross performance.

On average, pericarp traits were thicker for lines per se than the corresponding testcross hybrids (Table 3.5). Thinner pericarp detected in TP may be due to the additive and dominance effect of BH10 alleles in combination with the selected lines. It also may be due to the effect of creating a homozygous state for favorable QTL alleles of BH20 or BH30. However, the effects of homozygous state of favorable BH20 or BH30 allele may be less important because TP1 also showed reduced pericarp thickness despite BH1020 as the tester. The TP1evaluation has BH20 parental inbred in the testcross hybrid. The BH20 inbred has that has thicker pericarp and thus contributes more unfavorable alleles to the TC population. Therefore, the significant reduction of

pericarp thickness in TP appears to be due to mostly the effect of BH10 alleles. The difference between Group 1 and 2 was larger in LP than TP, and with a larger standard error. This is not surprising as use of a common tester generally reduces variation in hybrids as opposed to corresponding lines per se.

These results for pericarp thickness traits imply that both testers, BH1020 and BH1030, reduced pericarp thickness in TC population and also variability. The testcross populations are similar to backcross populations and they are expected to display less genetic and phenotypic variability than LP. Therefore, BH10 may not be the best tester to maximize genetic variation for pericarp thickness, but BH10 may be a good donor of favorable alleles for reducing pericarp thickness in hybrids, providing alleles that are not present in the BH20 and BH30 background.

The midparent value between the BH1020 tester and mean of selected MAS parental lines (MP1) was significantly larger than the mean of the TP1 for UG and CWN. For LA, MP1 was smaller than the mean of the TP1. The midparent value between BH1030 tester and mean of selected MAS parental lines (MP2) was significantly larger than the mean of the TP2 for all pericarp thickness traits (Table 3.5 and Appendix 3.4). All the significant differences demonstrated reducing pericarp thickness effects in TP, except in TP1 for LA (Table 3.5 and Appendix 3.4). This result may suggest that BH10 has some dominance effects when combined with BH30 and BH20 for reducing pericarp thickness in 3-way hybrids. The difference between the MP2 and TP2 (dominance effect of TP2) showed significantly larger difference compared to the difference between the MP1 and TP1 (dominance effect of TP1) for all pericarp thickness traits (Appendix 3.4). This suggests that BH1030 tester exhibited more dominance effect than BH1020 tester in this TC population evaluation.

Most of ear traits except NK and NR for TP1 and CL and KT for TP2 showed an increase in their values compared to LP, likely due to the heterosis effects of the three-way hybrids.

When the midparent value of TP1, MP1 and midparental value of TP2, MP2, were compared with TP1 and TP2 values for ear traits, we found significant positive dominance effects of both testers for ED and CD (Appendix 3.5). In addition, TP1 showed higher values in EW and TOTALKW compared to MP1, and TP2 showed lower values in NK and CL compared to MP2 (Table 3.6 and Appendix 3.5). This result suggests that BH1020 alleles had positive dominance effect in TC population for increasing EW and TOTALKW in TP1. In contrast, BH1030 alleles may have negative dominance effects in TP2 for decreasing NK and CL. The differences between MP1 and TP1 showed significantly larger values for CL, KD, NK, NR, EW, CW and TOTALKW than the differences between MP2 and TP2 (Appendix 3.5). Thus the BH1020 tester showed more positive effects on the traits in TP1 than the effects of BH1030 in TP2.

The phenotypic correlations between TP (includes both TP1 and TP2) and LP and between TP1 and TP2 were highly significant for all pericarp thickness traits (Appendix 3.6). This was somewhat expected because of high heritability observed for pericarp traits in the mapping population. This result may imply that selecting lines in the selfing generations based on per se QTL information on pericarp thickness traits may also result in simultaneous improvement of hybrid performance for pericarp thickness traits.

The correlations on ear traits between line per se and three-way hybrid evaluations were generally low to moderate. The CD trait showed significant correlations between LP and TP1 (0.39), between LP and TP2 (0.41), and between TP1 and TP2 (0.50). The correlations for KT and NR were positively significant between LP and TP1, and the correlations for CL and CW

were positively significant between LP and TP2 and between TP1 and TP2. However, the correlations between LP and TP were generally low on most of ear traits (Appendix 3.6).

Because LP and TP were grown in different environments, these comparison results between LP and TP may be confounded by to GxE interactions. Ear traits showed relatively low to moderate heritabilities and significant environmental effects in the mapping population, thus the results for ear traits are likely to be influenced by GxE interactions. In contrast, since heritabilities of pericarp thickness traits were relatively high, these comparisons may be more useful for further understanding of pericarp thickness trait performance in testcross population in comparison to $F_{2:3}$ population. These results are reported as preliminary exploratory results. More studies on the ear and pericarp traits involving both per se lines and corresponding testcross lines grown side by side are necessary to properly evaluate this type of comparison.

After evaluating each line in TC population, we could identify a few lines, 106-2, 107-1, 107-4 and 107-6 from TP2, that had thinner average pericarp thickness in three-way hybrids than grandparental inbreds and the hybrids: BH1020, BH1030 and BH2030 (Table 3.7). All selected lines were from subpopulation 6. Notably, 107-4 was selected as the best phenotypic line for pericarp thickness in subpopulation 6 in MAS population, and 106-2 was selected as the best genotypic line based on favorable QTL alleles in subpopulation 6. This shows some agreement between phenotypic and genotypic selection of per se lines and corresponding superior performance in a testcross hybrid.

SUMMARY AND CONCLUSIONS

From these studies it is apparent that pericarp thickness in three-way hybrids varies significantly for different regions within a single kernel, and differs some among positions within a single ear. This indicated that some aspects of phenotypic breeding for pericarp thickness may have difficulties due to experimental measuring errors. Selecting well-formed kernels and measuring a consistent region of kernels and ears are important considerations for accurate phenotypic measurement of pericarp thickness. This result indicates that MAS based on QTL estimation should be based on carefully collected phenotypic data sets.

Through the selection procedure based on QTL information for pericarp thickness traits, a group of promising lines with a high number of favorable alleles (Group 1) and a group of lines with a lower number of favorable alleles (Group 2) showed significant difference in both per se and testcross performance. These lines also showed high correlations between LP and TP, albeit confounded by environment. Therefore, early generation selection of QTL with additive effects for pericarp thickness traits may be promising for simultaneous indirect improvement of TP. Both BH1020 and BH1030 testers contributed to thinner pericarp with also favorable ear traits in TP, even though they were partially related to the selected lines testcrossed. Notably BH1030 tester showed more significant dominance effects than BH1020 tester so that thinner pericarp was observed in TP2 than TP1. Significant improvements on ear traits in hybrids were also achieved due to the possible heterosis effects. Studies in different environments are needed to evaluate the phenotypic trait performance in both LP and TP. Due to the additive and dominance effects of the testers, we could also detect some promising thinner-pericarped three-way hybrids compared to the parental selected lines and the testers (Table 3.7 and Figure 3.2). These will need

to be confirmed through further side-by-side comparisons. Through evaluating testcross performance, we were able to find some evidence that the QTL information could be utilized through MAS to reduce pericarp thickness while maintaining favorable ear traits important to fresh waxy corn hybrid breeding.

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TABLES AND FIGURES

Figure 3.1 Allele and genotype frequencies of the selected parental lines, testers and their F_1 three way hybrids.

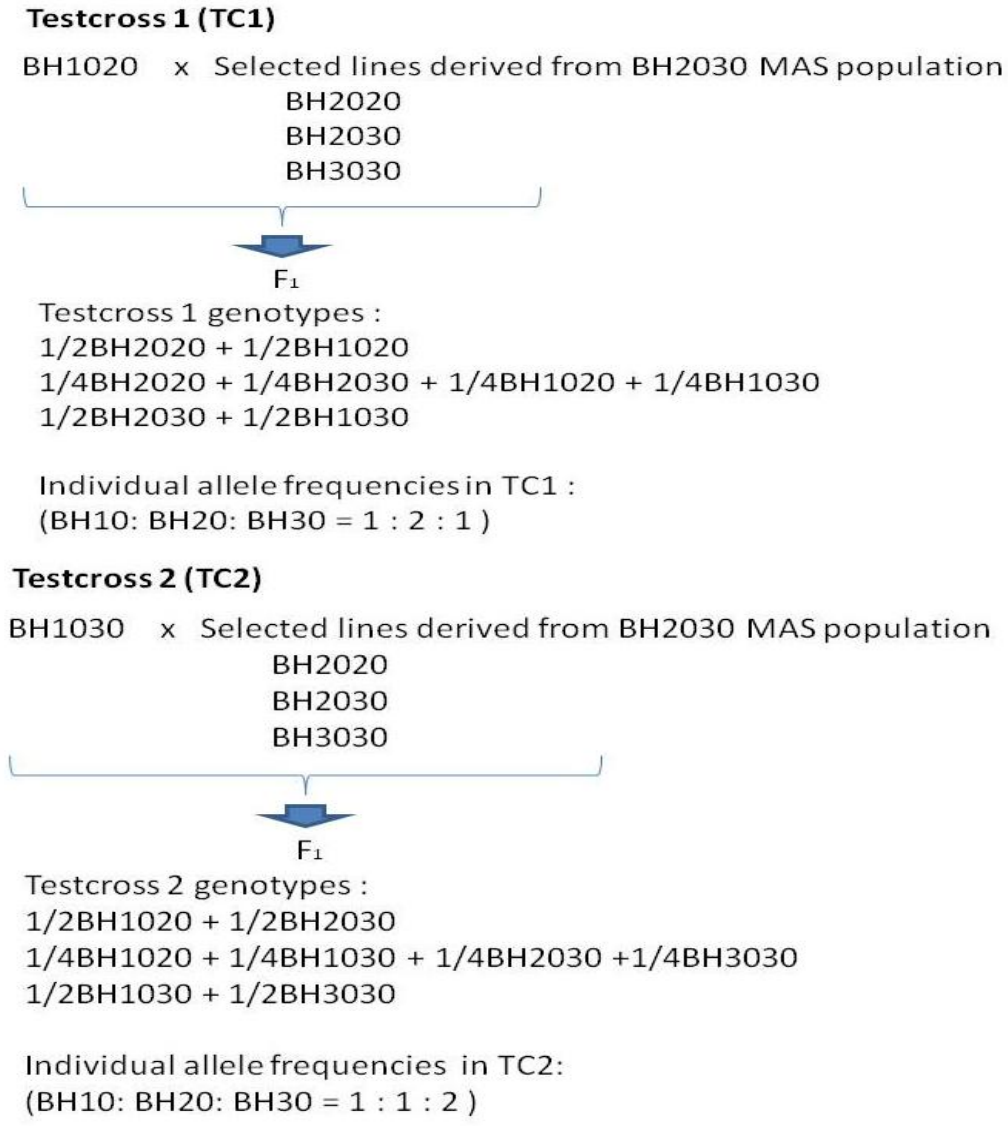


Table 3.2 Average proportions of favorable and unfavorable alleles for pericarp thickness QTL in Group 1 and 2 from MAS population.

	N	Percentage of homozygous favorable alleles (in %)	Percentage of homozygous unfavorable alleles (in %)	Percentage of heterozygote (in %)
Group 1	17	60	11	21
Group 2	13	29	47	18

Table 3.3 ANOVA table for pericarp thickness traits in TC population.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Upper germinal					
Replication	1	502.66	502.66	16.67	<.0001
Tester	1	753.63	753.63	24.99	<.0001
Group	1	770.32	770.32	25.55	<.0001
Ear Position	2	34.655	17.328	0.57	0.5635
Lower germinal					
Replication	1	730.22	730.22	15.20	0.0001
Tester	1	2382.80	2382.80	49.61	<.0001
Group	1	1009.10	1009.10	21.01	<.0001
Ear Position	2	1149.60	574.79	11.97	<.0001
Upper abgerminal					
Replication	1	193.86	193.86	4.72	0.0305
Tester	1	3612.90	3612.90	88.01	<.0001
Group	1	726.53	726.53	17.70	<.0001
Ear Position	2	64.835	32.42	0.79	0.4548
Lower abgerminal					
Replication	1	450.75	450.75	5.75	0.017
Tester	1	8255.10	8255.10	105.37	<.0001
Group	1	706.23	706.23	9.01	0.0029
Ear Position	2	891.06	445.53	5.69	0.0037
Crown					
Replication	1	117.35	117.35	5.07	0.025
Tester	1	1040.10	1040.10	44.91	<.0001
Group	1	728.72	728.72	31.47	<.0001
Ear Position	2	260.40	130.20	5.62	0.004

Table 3.4 Means of pericarp thickness traits for different ear regions in TC population.

	Middle	Top	Bottom
Upper germinal	39.03±0.55[§]a[%]	39.58±0.58 a	38.37±0.52 a
Lower germinal	49.89±0.86 a	52.14±0.89 b	54.11±0.87 c
Upper abgerminal	38.20±0.70 a	37.15±0.67 a	37.05±0.65 a
Lower abgerminal	47.26±1.00 a	47.65±1.02 a	50.63±1.06 b
Crown	31.21±0.46 a	30.14±0.43 ab	29.09±0.46 b

§ Standard errors are attached.

% Means within a column followed by the same letter is not different at the 0.05 probability level.

Table 3.5a Means of pericarp thickness traits for each group in MAS population.

	N	Upper germinal	Lower germinal	Upper abgerminal	Lower abgerminal	Crown
MAS (LP)						
Group 1	17	43.53±3.07§	55.20±3.08	41.96±3.92	52.35±4.86	33.83±2.94
Group 2	13	49.82±3.07	65.03±3.64	50.32±3.14	63.89±4.69	42.19±2.52
Group 1 & 2	30	46.26±2.23	59.46±2.48	45.58±2.68	57.35±3.53	37.45±2.11

§ Standard errors.

Table 3.5b Means of pericarp thickness traits for each group and tester in TC population.

	N	Upper germinal	Lower germinal	Upper abgerminal	Lower abgerminal	Crown
Testcross to BH1020 (TP1)						
Group 1	17	39.14±1.27§	52.28±1.75	39.28±1.74	51.58±2.27	30.77±1.18
Group 2	13	41.26±1.23	55.13±1.49	41.41±1.49	53.49±2.07	33.09±1.10
Group 1 & 2	30	40.06±0.90	53.52±1.19	40.20±1.18	52.40±1.55	31.77±0.84
Testcross to BH1030 (TP2)						
Group 1	15	35.27±1.02	46.37±1.38	31.92±1.11	40.71±1.36	26.64±0.95
Group 2	11	39.42±1.45	50.59±1.71	35.87±1.80	44.80±2.25	30.37±1.33
Group 1 & 2	26	37.03±0.93	48.15±1.14	33.59±1.00	42.44±1.27	28.22±0.85
Tester means and midparent values						
BH1020	6	36.38±1.51	48.56±2.93	35.53±2.04	43.35±2.45	29.08±1.30
BH1030	6	35.48±0.66	42.36±0.55	30.90±0.26	37.88±0.16	26.76±0.15
Midparent value of TP1		41.32	54.01	40.56	50.35	33.27
Midparent value of TP2		40.87	50.91	38.24	47.62	32.11

§ Standard errors.

Table 3.6a Means of ear traits for each group in MAS population (LP).

	CL	ED	CD	KD	KT	NK	NR
MAS (LP)							
Group 1	131.8±2.5§	32.9±0.6	20.8±0.5	12.0±0.7	4.8±0.1	24.4±0.9	10.4±0.2
Group 2	143.2±7.6	32.3±1.1	21.8±1.0	10.5±0.9	5.2±0.1	24.0±1.3	10.0±0.4
Group 1 & 2	136.3±3.6	32.6±0.6	21.3±0.5	11.4±0.6	5.0±0.1	24.2±0.7	10.2±0.2

	EW	CW	TOTALKW
MAS (LP)			
Group 1	50.6±3.4	6.9±0.4	43.7±3.2
Group 2	50.6±4.1	8.6±1.0	42.0±3.2
Group 1 & 2	50.6±2.6	7.7±0.5	43.0±2.2

§ Standard errors.
CL: Cob length, ED: Ear diameter, CD: Cob diameter, KD: Kernel depth, NK: Number of kernels/row, KT: Kernel thickness, NR: Number of rows/ear,
EW: Ear weight, CW: Cob weight, TOTALKW: Total kernel weight of ear.

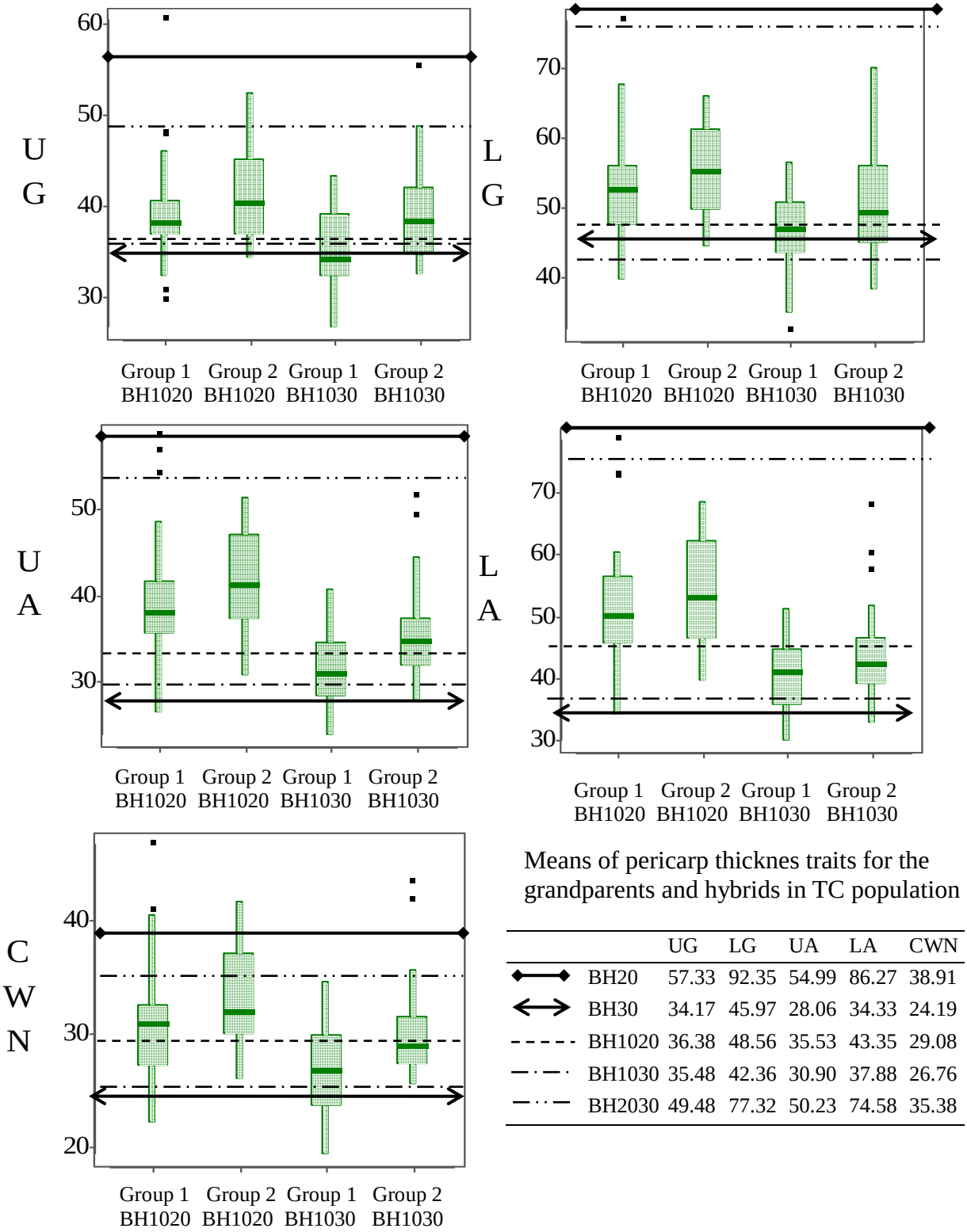
Table 3.6b Means of ear traits for each group and tester in TC population.

	CL	ED	CD	KD	KT	NK	NR
Testcross to BH1020 (TP1)							
Group 1	134.7±2.3§	34.8±0.4	21.8±0.3	12.9±0.4	4.9±0.1	23.3±0.4	9.7±0.1
Group 2	142.0±2.0	33.9±0.9	22.0±0.4	12.0±0.6	5.1±0.1	23.1±0.7	9.5±0.2
Group 1 & 2	137.9±1.7	34.4±0.5	21.9±0.2	12.5±0.3	5.0±0.0	23.2±0.4	9.6±0.1
Testcross to BH1030 (TP2)							
Group 1	131.4±2.6	36.4±0.5	23.1±0.3	13.3±0.4	4.6±0.1	24.5±0.5	11.0±0.1
Group 2	137.8±3.7	37.0±0.4	23.6±0.3	13.4±0.3	4.6±0.1	25.1±0.7	10.9±0.2
Group 1 & 2	134.1±2.2	36.7±0.3	23.3±0.2	13.3±0.3	4.6±0.0	24.7±0.4	11.0±0.1
Tester means and midparent values							
BH1020	134.5±5.8	33.7±2.2	21.6±1.5	12.1±1.0	5.2±0.1	21.9±1.2	8.8±0.4
BH1030	146.3±1.2	39.8±1.6	23.8±0.9	16.0±0.9	4.1±0.1	29.2±0.4	12.2±0.1
Midparent value of TP1	135.4	33.2	21.4	11.8	5.1	23.1	9.5
Midparent value of TP2	141.3	36.2	22.5	13.7	4.6	26.7	11.2

	EW	CW	TOTALKW
Testcross to BH1020 (TP1)			
Group 1	79.2±2.4	11.7±0.4	67.7±2.3
Group 2	74.6±2.9	11.8±0.3	62.9±2.8
Group 1 & 2	77.2±1.9	11.7±0.3	65.6±1.8
Testcross to BH1030 (TP2)			
Group 1	79.4±2.5	9.5±0.5	69.9±2.1
Group 2	82.9±2.7	9.7±0.5	73.1±2.3
Group 1 & 2	80.9±1.8	9.6±0.3	71.3±1.6
Tester means and midparent values			
BH1020	73.1±9.1	11.6±1.4	61.5±8.0
BH1030	109.2±2.5	11.2±0.2	98.0±2.3
Midparent value of TP1	61.9	9.7	52.2
Midparent value of TP2	79.9	9.4	70.5

§ Standard errors.
CL: Cob length, ED: Ear diameter, CD: Cob diameter, KD: Kernel depth, NK: Number of kernels/row, KT: Kernel thickness, NR: Number of rows/ear,
EW: Ear weight, CW: Cob weight, TOTALKW: Total kernel weight of ear.

Figure 3.2 Boxplots of pericarp thickness traits for each group and testers in TC population.



Means of pericarp thicknes traits for the grandparents and hybrids in TC population

	UG	LG	UA	LA	CWN
BH20	57.33	92.35	54.99	86.27	38.91
BH30	34.17	45.97	28.06	34.33	24.19
BH1020	36.38	48.56	35.53	43.35	29.08
BH1030	35.48	42.36	30.90	37.88	26.76
BH2030	49.48	77.32	50.23	74.58	35.38

Table 3.7a Best lines based on pericarp thickness phenotypes from TC population.

Source	Group	Tester	Upper germinal	Lower germinal	Upper abgerminal	Lower abgerminal	Crown
106-2	1	BH1030	27.66	33.79	25.16	31.09	20.75
107-1	1	BH1030	31.55	40.69	26.50	34.25	22.15
107-6	1	BH1030	32.97	41.63	27.38	35.43	23.73
107-4	1	BH1030	32.43	43.03	28.80	37.47	23.63

Table 3.7b Per se performance of best lines identified based on pericarp thickness phenotypes from TC population.

Source	Group	Population	Upper germinal	Lower germinal	Upper abgerminal	Lower abgerminal	Crown
106-2	1	MAS	28.4	36.4	31.4	36.4	25.2
107-1	1	MAS	32.4	41.8	30.0	36.4	26.6
107-6	1	MAS	34.6	42.0	31.2	38.0	28.8
107-4	1	MAS	31.5	50.75	24.75	32.5	20.5

APPENDIX

Appendix A.1 Genotypic data on selected markers of pericarp thickness QTL for the lines selected for the testcross from MAS population.

Source	Group	Sub-population[a]	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15	M16
13.2	Group1	1	2[b]	1	2	2	0	2	0	2	2	0	2	2	0	1	.	0
14.2	Group1	1	2	2	2	1	0	2	2	2	2	2	2	2	1	.	.	.
14.5	Group1	1	2	2	2	1	2	2	2	2	1	1	2	2	1	1	1	.
15.2	Group1	1	2	1	2	2	2	2	2	2	2	2	2	1	1	2	2	.
15.3	Group1	1	2	1	2	1	1	2	2	2	2	0	2	1	1	1	2	.
26.4	Group1	4	2	2	2	0	2	2	2	0	1	0	2	1	1	1	2	.
26.5	Group1	4	2	2	2	1	2	2	2	0	1	1	2	.	1	.	1	.
27.3	Group1	4	2	1	2	2	2	2	0	0	2	1	2	.	1	2	2	.
29.3	Group1	4	2	2	2	1	2	2	2	0	1	2	2	1	1	1	2	.
75.3	Group1	4	0	2	2	0	2	2	2	2	1	1	2	2	1	2	2	.
75.5	Group1	4	2	2	2	2	2	2	2	1	1	0	2	1	1	2	2	.
106.2	Group1	6	.	.	2	2	0	1	2	2	2	2	2	2	2	2	2	1
107.1	Group1	6	2	2	2	1	0	2	.	.	2	1	2	2	0	2	2	0
107.4	Group1	6	2	2	2	2	0	0	0	2	1	2	2	2	2	2	2	2
107.6	Group1	6	2	0	2	1	2	2	2	2	2	1	2	2	2	2	2	1
120.1	Group1	8	.	.	2	1	1	2	0	1	2	0	2	2	.	2	2	.
123.1	Group1	8	2	2	1	2	0	2	2	2	1	0	2	2	2	2	2	0
17.4	Group2	1	2	1	2	0	2	1	1	0	0	1	1	0	0	1	0	.
21.2	Group2	1	2	2	0	0	1	2	2	0	0	0	0	1	1	0	0	.
21.3	Group2	1	2	2	0	0	0	2	2	0	1	0	0	0	2	0	0	.
44.3	Group2	2	2	2	2	2	.	1	0	0	0	2	2	0	0	0	0	1
50.2	Group2	2	2	0	2	1	0	2	0	1	1	0	2	1	0	0	0	.
54.5	Group2	3	2	2	0	0	2	1	0	1	1	0	0	0	1	0	2	.
30.3	Group2	4	2	2	2	0	2	2	0	0	1	0	2	0	0	0	0	.
73.3	Group2	4	1	0	1	2	2	2	1	0	0	2	2	0	1	0	1	0
129.1	Group2	5	0	0	0	0	2	2	0	0	0	1	2	2	0	0	0	.
105.1	Group2	6	2	2	0	1	0	0	0	2	2	0	0	2	0	2	2	1
130.2	Group2	7	0	0	0	0	0	2	0	1	0	1	2	1	.	2	2	1
130.5	Group2	7	2	2	0	0	0	2	.	1	0	0	2	0	0	0	1	0
133.1	Group2	7	0	2	2	0	0	2	.	0	0	0	2	1	2	0	1	.

a Each selected line was belong to the subpopulation in MAS population.

b 0, 1 and 2 denoted as homozygous unfavorable allele, heterozygous, and homozygous favorable allele for pericarp thickness QTL respectively.

Appendix A.2 ANOVA table for ear traits in TC population.

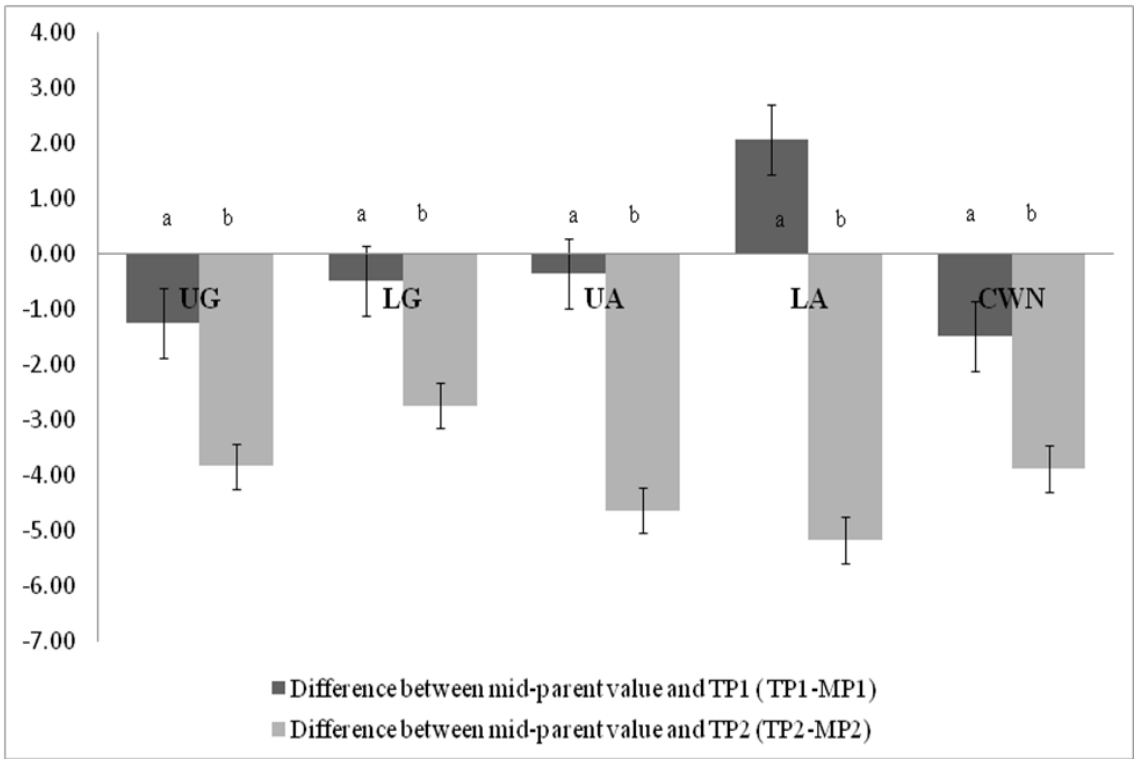
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Cob length					
Tester	1	389.67	389.67	2.81	0.0966
Group	1	1260.81	1260.81	9.09	0.0032
Tester * Group	1	6.58	6.58	0.05	0.8280
Ear diameter					
Tester	1	147.87	147.87	15.09	0.0002
Group	1	0.77	0.77	0.08	0.7793
Tester * Group	1	14.91	14.91	1.52	0.2202
Cob diameter					
Tester	1	55.68	55.68	15.87	0.0001
Group	1	3.12	3.12	0.89	0.3475
Tester * Group	1	0.79	0.79	0.23	0.6358
Kernel depth					
Tester	1	23.08	23.08	5.21	0.0244
Group	1	5.40	5.40	1.22	0.2720
Tester * Group	1	6.93	6.93	1.56	0.2138
Kernel thickness					
Tester	1	4.58	4.58	67.06	<.0001
Group	1	0.41	0.41	5.93	0.0165
Tester * Group	1	0.21	0.21	3.14	0.0791
Number of kernels/row					
Tester	1	67.94	67.94	10.56	0.0015
Group	1	1.22	1.22	0.19	0.6646
Tester * Group	1	4.36	4.36	0.68	0.4122
Number of rows/ear					
Tester	1	50.93	50.93	85.64	<.0001
Group	1	0.92	0.92	1.55	0.2162
Tester * Group	1	0.25	0.25	0.42	0.5205
Ear weight					
Tester	1	479.79	479.79	3.39	0.0684
Group	1	7.98	7.98	0.06	0.8129
Tester * Group	1	439.50	439.50	3.10	0.0810
Cob weight					
Tester	1	123.99	123.99	30.21	<.0001
Group	1	1.08	1.08	0.26	0.6082
Tester * Group	1	0.11	0.11	0.03	0.8705
Total kernel weight/ear					
Tester	1	1061.34	1061.34	9.26	0.0029
Group	1	18.72	18.72	0.16	0.6869
Tester * Group	1	444.96	444.96	3.88	0.0514

Appendix A.3 Means of pericarp and ear traits on parental inbred lines and their hybrids, BH1020, BH1030 and BH2030 measured from 2008 winter nursery.

	UG	LG	UA	LA	CWN	CL	ED	CD	KD	KT	NK	NR	EW	CW	TOTAL KW
BH20	57.33	92.35	54.99	86.27	38.91	115.00	31.58	21.43	10.15	5.75	16.00	8.05	28.70	7.58	21.12
BH30	34.17	45.97	28.06	34.33	24.19	87.25	27.67	20.67	7.00	4.39	19.08	13.00	23.38	3.08	20.31
BH1020	36.38	48.56	35.53	43.35	29.08	134.48	33.72	21.59	12.13	5.15	21.93	8.84	73.12	11.67	61.45
BH1030	35.48	42.36	30.90	37.88	26.76	146.27	39.80	23.83	15.97	4.13	29.17	12.20	109.19	11.18	98.01
BH2030	49.48	77.32	50.23	74.58	35.38	130.40	40.85	23.50	17.35	4.56	23.45	10.75	92.71	11.16	81.55

UG: Upper germinal, LG: Lower germinal, UA: Upper abgerminal, LA: Lower abgerminal, CWN: Crown,
CL: Cob length, ED: Ear diameter, CD: Cob diameter, KD: Kernel depth, NK: Number of kernels/row, KT: Kernel thickness, NR: Number of rows/ear, EW: Ear weight, CW: Cob weight, TOTALKW: Total kernel weight of ear.

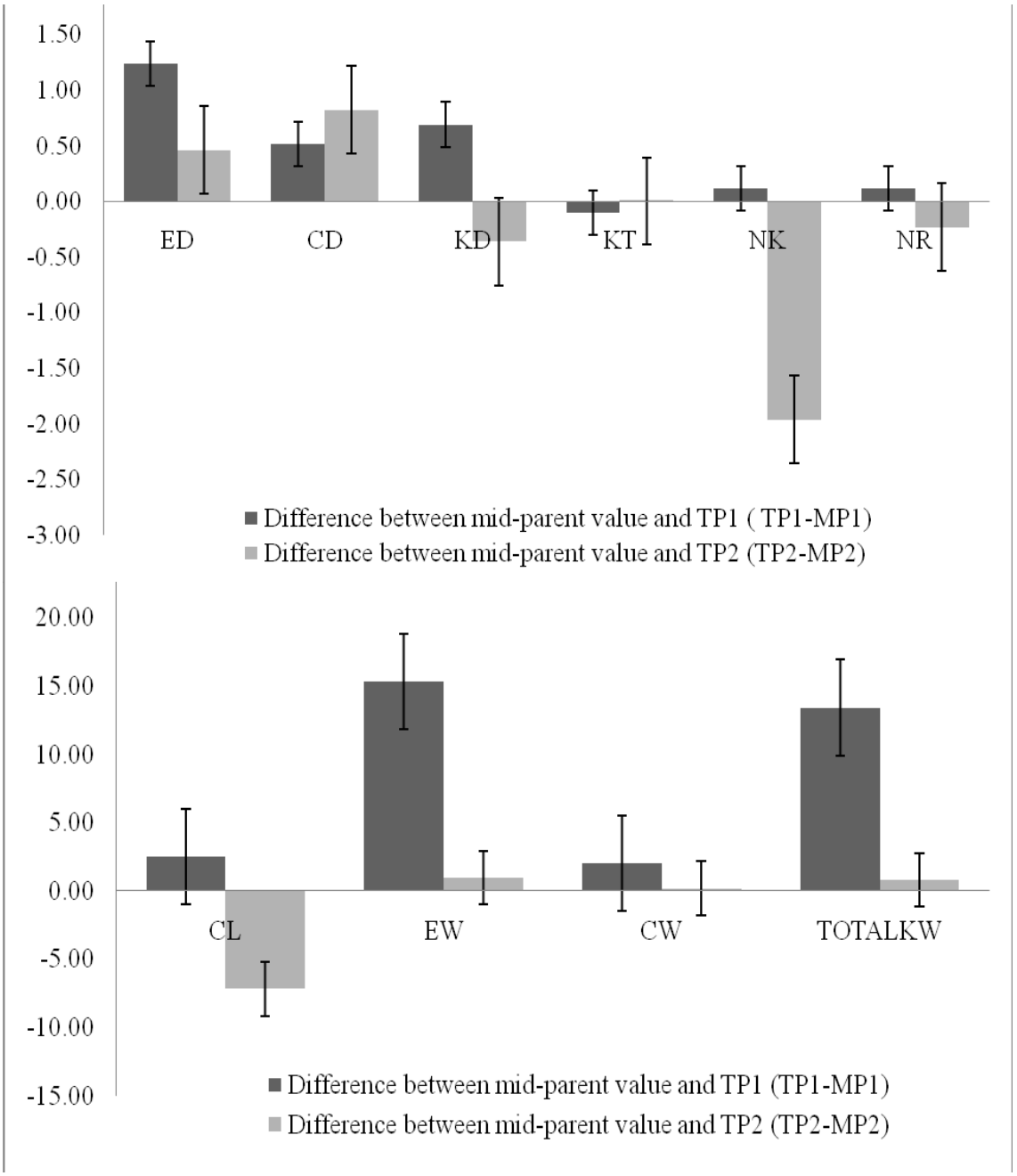
Appendix A.4 Differences between the TP and the corresponding midparent values for pericarp thickness traits in TC population.



The different letters on the columns within the traits show the significant difference at the 0.05 probability level.

UG: Upper germinal, LG: Lower germinal, UA: Upper abgerminal, LA: Lower abgerminal, CWN: Crown.

Appendix A.5 Differences between the TP and the corresponding midparent values for ear traits in TC population.



CL: Cob length, ED: Ear diameter, CD: Cob diameter, KD: Kernel depth, NK: Number of kernels/row, KT: Kernel thickness, NR: Number of rows/ear,
EW: Ear weight, CW: Cob weight, TOTALKW: Total kernel weight of ear.

Appendix A.6 Phenotypic correlations for each trait between line per se performance (LP) and testcross performance (TP1 and TP2), and between the tester BH1020 (TP1) and the tester BH1030 (TP2).

Traits	LP vs. TP1	LP vs. TP2	TP1 vs. TP2
Pericarp thickness traits			
Upper germinal	0.76**	0.77**	0.68**
Lower germinal	0.71**	0.72**	0.65**
Upper abgerminal	0.78**	0.75**	0.68**
Lower abgerminal	0.81**	0.73**	0.60**
Crown	0.77**	0.75**	0.70**
Ear traits			
Cob length	0.23	0.40*	0.54**
Ear diameter	0.22	-0.06	0.35
Cob diameter	0.39*	0.41*	0.50**
Kernel depth	-0.00	-0.03	-0.03
Kernel thickness	0.39*	0.34	0.29
Number of kernels/row	0.09	-0.01	0.23
Number of rows/ear	0.43*	0.04	0.28
Ear weight	0.07	-0.07	0.08
Cob weight	0.29	0.39*	0.54**
Total kernel weight	0.09	-0.15	0.05

*, ** Significant at P<0.05 and P<0.01, respectively.