ABSTRACT

TARTER, JENNIFER ANN. Additive genetic effects of single chromosomal segment introgressions and epistatic effects of paired introgressions on quantitative traits in maize (*Zea mays* L.). (Under the direction of James B. Holland and Major M. Goodman). Epistasis effects estimated in conjunction with quantitative trait locus (QTL) mapping are often confounded by segregating background genomes or QTL × background interactions. To improve the precision of estimating QTL epistatic effects, 16 near-isogenic lines (NILs), resulting from the introgression of chromosomal segments of Tx303 into the genetic background of inbred line B73, were crossed in all pairwise combinations. Marker-assisted selection and self-fertilization were employed to create double-introgression NILs (dNILs) homozygous for two introgressed segments. The resulting 127 dNILs, their 16 parental, single-introgression NILs, and inbred lines B73 and Tx303 were evaluated as inbred lines, *per se*, as well as in testcross combinations with Mo17, in replicated field trials to measure the effects of introgressions singly (additive effects) and in pairs (epistatic effects). Across traits tested, significant additive effects involving single NILs were detected in 2% to 28% of the tests in the inbred trials and between 2% and 40% of the tests involving single NILs in testcross in the hybrid trials. Significant epistatic effects were identified in 2% to 19% of the tests including inbred dNILs and in 2% to 27% of the tests including testcross dNILs. Generally, the frequency and magnitude of epistatic interactions were less than those of additive effects for both the inbred and hybrid trials. Across traits, between 0% and 100% of the significant epistatic interactions involved chromosomal segments that did not display independent significant additive effects. There was minimal congruency between

chromosomal regions displaying significant genetic effects in the inbred and hybrid trials. These results suggest that epistatic interactions can affect predictions of phenotypic performance based solely on additive effects detected either in inbred lines or hybrid combinations. Such predictions might be biased and warrant testing in both population types.

ADDITIVE GENETIC EFFECTS OF SINGLE CHROMOSOMAL SEGMENT INTROGRESSIONS AND EPISTATIC EFFECTS OF PAIRED INTROGRESSIONS ON QUANTITATIVE TRAITS IN MAIZE (*Zea mays* L.)

by

JENNIFER ANN TARTER

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APPROVED BY :

Dr. James B. Holland
Co-chair of Advisory Committee

Dr. Major M. Goodman
Co-chair of Advisory Committee

Dr. J. Paul Murphy

Dr. Jason A. Osborne

BIOGRAPHY

Jennifer Ann Tarter was born in the small town of Breese, Illinois in the terrible winter of 1977, the eldest child of Bill and Barbara Tarter. After spending countless summers doing crop research with the family agribusiness firm Alvey Labs, Inc., and becoming a senior staff member at the age of 18, she moved to Champaign-Urbana and attended the University of Illinois College of Agricultural, Consumer, and Environmental Sciences.

During her time at the U of I, Jennifer was very active with the undergraduate division of the American Society of Agronomy and served as Vice President in 1998-1999. She was recognized as a J.B. Tuner scholar during her tenure in Champaign and was also named a Warren K. Wessels finalist in May 1999. After spending one summer with a molecular biology internship, Jennifer realized her interest in traditional field breeding and began her search for a graduate program. Jennifer graduated with a Bachelor's of Science in Crop Sciences with High Honors in May 1999.

Jennifer then moved to North Carolina and joined Dr. Jim Holland with the USDA Maize Phenomics program and learned, among numerous other things, that it is possible to grow corn in soil, clay-brick, sand, and even rocks. She spend two and one-half years studying semitropical populations of maize under the direction of Jim Holland and Major Goodman and was awarded a Masters of Science degree in May 2002 for her tenacity. Jennifer then switched gears and began a labor-intensive program to develop populations of maize to answer some long-ignored fundamental questions of gene interaction. After over three years of hard work, the epistasis project was completed and Jennifer sucessfully defended her Ph.D. in March 2005. She's now moving to north-

central Iowa to join Pioneer Hybrids in a research position with the maize inbred discovery team.

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I. Literature Review

Definition and examples of epistasis

Bateson (1907) coined the term epistasis to describe the interaction of genetic factors as observed in their joint effects on phenotypes. In modern quantitative genetics, epistasis refers more generally to the interaction of alleles at different loci which results in phenotypic values not predicted by the sum of individual locus effects, and is a form of non-additive gene action. The simplest epistatic interactions are duplicate and complementary interactions. Duplicate interactions occur when two or more loci perform the same function. If at least one of the loci is functioning, the gene product will be produced. The failure of one locus to produce its product will not result in an abnormal phenotype. Hatchett *et al.* (1993) reported duplicate epistasis between two genes conditioning antibiosis to first-instar Hessian fly (*Mayetiola desctructor* (Say)) larvae in rye (*Secale cereale* L.). Complementary interactions result when more than one gene is necessary for the final product. For example, if two or more genes are involved in a biosynthetic pathway, both genes must be functioning and their products must be present in order to produce the final product. If either gene product is absent, the pathway will cease to function and the final product will not be produced. Paris (2002) reported complementary epistasis between the effects of two genes on fruit color and striping of *Cucurbita pepo* L. Ahmadi *et al.* (2001) confirmed complementary epistasis between two *Rice yellow mottle virus* quantitative trait loci in rice (*Oriza sativa* L.) required to confer disease resistance.

Uncertainty in terminology regarding epistasis often arises because of the differences in definitions and types of epistasis being reported. The first division occurs between the two major fields of genetics: classical genetics and statistical genetics.

Classical, or Mendelian, genetics refers to epistasis strictly as the masking of the effects of one locus by another; whereas statistical, or biometrical, genetics considers epistasis as any form of non-allelic interaction (Phillips 1998). These distinctions stem from nearly 100 years of philosophical differences between researchers focused on Mendelian segregation ratios and those studying the biometrical laws of heredity (Phillips 1998). The second area of confusion lies within the distinctions in nomenclature relating to test subjects used to measure epistasis. Statistical epistasis considers the structure of the test population and its gene frequencies in the definition of epistasis. In contrast, physiological epistasis is defined solely on the basis of genotype values, without regard to their population frequencies (Cheverud and Routman 1995). Physiological epistasis plays an important role in genetic variances because of its contributions to additive and dominance genetic variance components in defined populations (Crow and Kimura 1970). Cheverud and Routman (1995) suggested that the contributions of physiological epistasis to an individual's breeding value and thus to the additive genetic variance could be important, especially when considering evolutionary species models.

Epistatic effects have been subdivided into distinct classes based on individual gene action. Cockerham (1954; 1956) and Kempthorne (1954) partitioned epistatic effects into additive×additive, additive×dominance, and dominance×dominance effects based on genetic variance components. Lynch and Walsh (1997) compared the separation of epistatic effects to the partitioning of sums of squares from an analysis of variance. Each component of the additive main effects and epistatic effects (additive, dominance, additive×additive, additive×dominance, and dominance×dominance) can be isolated from the overall genetic model and an appropriate variance component assigned

to it. In a study of epistatic effects for body-weight in mice, Routman and Cheverud (1997) noted that loci involved in strong additive-by-additive epistasis were less likely to be identified as quantitative trait loci (QTL) for their trait of interest. The additive-by-additive epistasis results in "epistatic nullification" of quantitative loci which might reduce the number of QTL identified in an experiment.

Epistasis can be separated into several distinct classes. Synergistic epistasis occurs when epistatic effects occur in the same direction as the additive effects, i.e. when the effects of positive alleles at different loci are exaggerated when combined in the same genotype (Crow 1970). Synergistic epistasis can be positive or negative, depending on which alleles are considered "wild type". A synergistic response which leads to an increase in plant height or grain yield would be a welcome situation, in some cases. However, de Visser *et al.* (1997) reported synergistic epistasis between deleterious mutations that affected the carrying capacity of *Chlamydomonas* cultures. Synergistic epistasis of deleterious alleles provides the best evidence in favor of the mutational deterministic hypothesis of sexual reproduction (Elena and Lenski 1997). The mutational deterministic hypothesis maintains that sexual reproduction within species occurs to provide an opportunity for deleterious alleles to be grouped together in an individual, produce a negative phenotype, and thus be culled from the population.

Antagonistic epistasis occurs when the epistatic effects occur in the opposite direction of the two additive effects (Crow and Kimura 1970). Eshed and Zamir (1996) reported less-than-additive, or antagonistic epistatic interactions, between QTL demonstrating additive effects in the same direction that affected five yield traits in tomato (*Lycopersicon esculentum* L.). I suggest the term "non-canceling epistasis" to

refer to the situation when the additive effects of parental introgressions into a standard genetic background are in opposite directions for the trait of interest, and yet significant epistatic effects are observed when the introgressions are combined because the additive effects fail to cancel each other out. However, non-canceling epistasis can be considered a variant of either synergistic or antagonistic epistasis, depending on genotype designations. These three epistatic categories are defined by the traits and population being tested, the directions of the loci's effects, and the relationships between those specific gene effects.

Epistasis has also been categorized based on gene actions of the loci involved as opposed to directional effects, as discussed above. Fasoula and Fasoula (1997) separated epistatic effects into four types 1) positive epistasis – the overshadowing effect of one gene over other nonallelic genes, 2) semiepistasis – the additivity of nonallelic genes, 3) coepistasis – complementary gene action (two or more functioning genes necessary for trait expression), and 4) negative epistasis – nonallelic gene masking (gene expression inhibition). However, these terms are rarely applied to epistatic interactions reported in the literature.

Epistasis and Evolutionary Theories

Fisher (1918) and Wright (1921) developed contrasting theories of the evolutionary fate of organisms, as well as their fitness landscapes, based on differing views of the importance of epistasis. The first major difference between the two theories is the description of populations. Fisher considered populations to be large and panmictic (allowing genes to be tested in all genetic backgrounds) whereas Wright considered

species to be comprised of small subpopulations, or local populations. Fisher's (1930) theory maintains that evolution is due to selection acting on individual loci, independent of the effects of other loci. Therefore, average additive effects of individual genes are considered rather than gene combinations. Fisher adopted a 'target model of adaptation' which modeled the effects of mutations on population adaptation. The model included how the effectiveness of an individual mutation depends on where in the adaptation process it occurs and how that mutation could later translate into additive genetic effects (Brodie 2000).

In contrast, Wright (1931) developed the shifting balance theory (SBT) that relates the fitness peaks and valleys of an adaptive landscape to epistatic gene action. Wright (1968) later explained that natural selection operates on whole organisms, or genotypes, not on individual genes. Small subpopulations are able to move through fitness "valleys" by means of genetic drift whereupon rare favorable epistatic allele combinations can be assembled by chance. Fisher believed that selection on individual alleles in a sequential manner was the predominant force in species adaptation, while Wright maintained that selection acted simultaneously on multiple loci, allowing for selection of various allelic combinations (Brodie 2000). Coyne *et al.* (2000) argued against Wright's shifting balance theory citing the failure of the basic assumptions and components of the SBT theory (e.g., group selection, epistasis, subdivided populations) to hold up under natural conditions. However, Goodnight and Wade (2000) continued the debate begun by Fisher and Wright by countering the arguments of Coyne *et al.* (2000) and identifying the shortcomings in the opposing evolutionary theory, Fisher's large population size theory (LST).

Current biological fitness theories are attributing more and more importance to the concept of epistasis. Many critical biological processes have been reported to be significantly affected by epistasis, including the evolution of sex, diploid life cycles, mating systems, phenotypic plasticity, and developmental homeostasis (Fenster *et al.* 1997; Whitlock *et al.* 1995). Holland (2001) presented three implications for the presence of strong epistasis in plant breeding: epistatic variance can shift to additive variance under inbreeding, epistatic variance contributes to a "temporary" response to selection which can be captured as heterosis, and, finally, fitness, or crop yield, is not a simple function of allele frequencies, but represents a variable adaptive landscape with local fitness peaks and valleys.

Problems with previous epistasis studies

The relatively small magnitude of epistasis, compared to additive gene effects, has lead to limited study and understanding of epistasis. According to Whitlock *et al*. (1995) there are four basic problems with the measurement of epistasis in current experimentation. The four problems can be summarized as the use of the least squares statistical method of analysis of variance, the large confidence limits associated with epistatic effects, genotype-by-environment interactions, and finally, linkage disequilibrium.

First, the least squares statistical method commonly used to analyze breeding experiments assigns as much variation as possible to the main effects. This leaves little variation to be assigned to the interaction term or the epistatic effects. In agreement with Whitlock's report, Purcell and Sham (2004) also concluded that epistasis was difficult to

detect due to most of the experimental variation being assigned to main effect factors. Goodnight and Wade (2000) suggested that, in order to develop good tests for epistasis, a statistical model should be built first based on genic interactions, from which the genic main effects can later be derived.

A second problem with current experiments is that the confidence limits on epistatic effects estimates tend to be large (Whitlock *et al.* 1995). Other research also indicates that the magnitude of epistatic effects and their power of detection represent problems with current epistasis studies. Frankel and Schork (1996) attributed small sample size (only 25% of the individuals in a two locus system with equal allele frequencies have the potential to display a specific epistatic phenotype) and low statistical power for detection of epistasis as difficulties in practical biological studies. Finally, because the evaluation of each experimental line involves averaging over all other loci in the genotype, the epistatic effects are more similar to statistical averages than epistatic estimates (Phillips *et al.* 2000).

The final two problems with epistasis studies, as identified by Whitlock *et al.* (1995), are associated with genotype-by-environment interactions and linkage disequilibrium. The artificial testing environments used for these studies could promote interactions between genotype and environment, potentially eliminating the identification of genic interactions. Finally, linkage disequilibria, e.g., tight linkages of coadapted gene complexes, could disguise epistatic interactions between two genes. If the recombination frequency between the two genes is so low that recombination rarely occurs, it becomes nearly impossible to test the main effect of either gene without the confounding presence

of the second gene. Frankel and Schork (1996) also reported that the additive effects of physically linked genes might confound the detection of pure epistasis.

Several other issues tend to present problems when testing for epistatic effects. The influence of segregation in the genetic background may affect the phenotypic response of the two genomic regions of interest and thus bias the experimental results. Experimental design may influence the rates of epistasis detection. If each entry in an experiment is usually quite genetically dissimilar, the significant differences detected between entries due to the lack of genetic uniformity (Fasoulas and Allard 1962) might overshadow and prevent the detection of epistatic effects. Melchinger (1987) noted that positive and negative epistatic effects may cancel each other, with only net epistasis being measured in testcross studies; this may not allow for the detection of much epistasis. Routman and Cheverud (1997) maintained that "the failure … to find epistasis does not mean that physiological epistasis does not occur".

Epistatic Effects in Biometrical Studies

Despite the controversy about the importance of epistasis, significant epistatic effects have been reported for a number of species for at least 50 years. Spickett and Thoday (1966) demonstrated epistatic effects for bristle number in crosses between two inbred lines of *Drosophila melanogaster*. Joshi and Ugale (2002) reported high level epistatic interactions in pearl millet (*Pennisetum glaucum* (L.) R. Br.) with both digenic and trigenic models for resistance to downy mildew [*Sclerospora graminicola* (Sacc.) Schrot]. Johnson and Gepts (2002) reported significant epistatic effects for seed yield characters in recombinant inbred populations derived from a wide cross of common bean

(*Phaseolus vulgaris* L). They also showed that epistatic interactions, attributed to interactions between chromosomal linkage blocks from the two parents, were not responsible for poor hybrid performance in self-pollianted crops, as is sometimes suggested. Epistatic interactions have been reported for daylength response in soybean (*Glycine max* L.) (Fasoula *et al.* 1995; Saidon *et al.* 1989), protein and oil contents in peanut (*Arachis hypogea* L.) (Upadhyaya and Nigam 1999), and complex traits such as total glycoalkaloid content in tetraploid potato (*Solanum tuberosum* L.) (van Dam *et al.* 2003).

Maize (*Zea mays* L.) has been a model organism for genetic testing for decades, and the study of epistasis is no exception. Stuber *et al.* (1973) reported epistatic effects, of limited importance, for ear number, ear height, and plant lodging in a set of maize lines. In a study involving six generations (P1, P2, F_2, F_2-Syn8, BCP_1, and BCP_2) of maize material, Lamkey *et al.* (1995) reported significant epistatic effects for both grain yield and grain moisture, and 21% and 18% of the variability in those traits, respectively, was accounted for by epistasis. Wolf and Hallauer (1997) used the triple testcross design with a cross between B73 and Mo17 to detect and quantify the epistatic interactions involved in the agronomic performance of several traits in maize. They reported significant and stable epistatic interactions for several ear characteristics as well as flowering time traits. In these reports, epistatic interactions were detected more frequently on less complex traits (i.e. ear length, kernel-row, etc) than more complex traits, such as grain yield and grain moisture. This trend is not surprising, considering the number of genes involved in the less complex traits, and therefore the potential ease in

detecting interactions among them, as opposed to detecting interactions among the hundreds to thousands of genes involved in grain yield.

Flowering time has often been a subject of epistasis studies, perhaps because epistasis is more likely to be detected in less complex traits. Koornneef *et al.* (1998) developed double-mutant lines of *Arabidopsis thaliana* and reported significant epistatic interactions between several flowering time genes, which corresponded to the physiological flowering time groups that were previously assigned. McKay *et al.* (2003) also reported pleiotropic effects in *A. thaliana* lines that were developed with differing combinations of two flowering time genes.

Despite the previous evidence, epistatic effects are often ignored or found to be of little importance (Austin and Lee 1998; Bernacchi *et al.* 1998; Edwards *et al.* 1987). Hinze and Lamkey (2003) reported that epistasis for grain yield was not as prevalent as expected across a range of populations. They attributed the failure to detect epistasis to several conditions, including cancellation of negative and positive effects, inability to detect higher order epistatic interactions, and finally to poor testing conditions, which reflect three of the four problems with epistasis studies identified by Whitlock *et al.* (1995). Estimation techniques that are currently being used, such as estimatation of genetic variance components, are not very powerful for detecting epistasis (Hallauer and Miranda 1981).

QTL exhibiting epistasis

Quantitative trait loci can sometimes behave in an epistatic manner and be affected by the genetic background. Doebley *et al.* (1995) reported epistatic interactions

in maize between two QTL for inflorescence architecture as well as between the individual QTL and the maize or teosinte background genome. Additional studies have also reported epistasis among QTL for various traits in other species including aluminum tolerance in rice (Wu *et al.* 2000), oil content in rapeseed (*Brassica napus* L.) (Zhao *et al.* 2005), and multiple traits in tomato (Monforte *et al.* 2001). Yu *et al.* (1997) reported that epistatic interactions played a substantial role in QTL for grain yield in rice and may help to explain the genetic basis of heterosis in rice. Kim and Rieseberg (2001) reported that epistatic interactions influenced the genomic areas associated with pollen sterility QTL that were introgressed from wild into domesticated sunflower species (*Helianthus spp.*). Finally, Zhu *et al.* (2003) identified four QTL associated with tolerance to barley yellow dwarf virus in oat (*Avena sativa* L.), and they reported epistatic interactions between two different pairs of QTL that explained approximately 8% of the variation.

Problems with QTL mapping

Epistasis is often tested by measuring QTL × QTL interactions. However, Kim and Rieseberg (2001) noted that QTL mapping is biased against the detection of epistasis because of the potential differences in magnitudes of QTL main effects versus epistatic effects. Furthermore, several problems exist in standard QTL mapping that are exacerbated in QTL×QTL epistasis studies. The sample size within an experiment has a significant effect on the number of QTL identified in a given population (Beavis 1994; Melchinger *et al.* 1988). Beavis (1994) also suggested that the magnitude of the QTL main effects is affected by the number of progeny tested. The more progeny evaluated,

the greater the number of QTL detected, and the less bias in estimates of the genetic effects associated with the QTL. Beavis (1994) and Melchinger *et al.* (1988) suggested that congruency between QTL studies can be increased with additional sampling for QTL estimates. Usually, QTL are mapped and their effects estimated in the same population. This procedure usually leads to the overestimation of the QTL effects (Lande and Thompson 1990). Melchinger *et al.* (1998) reported that, in most cases, QTL effects were considerably smaller when estimated from an experiment different from the QTL mapping project.

One of the problems with the epistasis studies mentioned above was segregating background effects relative to the genomic regions of interest. This might be a plausible explanation for the variability of QTL acting in an epistatic manner. Orf *et al.* (1999) detected epistatic effects among quantitative trait loci for agronomic traits in soybean that depended on the parental allele present and the specific recombinant inbred population. Coaker and Francis (2004) reported additive-by-additive epistasis between two disease resistance QTL in tomato that heightened resistance to bacterial canker when present in the same genetic background. Lecomte *et al.* (2004) reported variability among fruit traits in tomato that were attributed to epistatic interactions between QTL and the genetic backgrounds. It is evident that the QTL effects are dependent not only on their own allelic makeup, but also on that of undetected QTL, or the background genome. Data such as these move to support Wright's theory of selection on genetic combinations, rather than merely individual loci.

Several other problems are also associated with QTL detection and estimation. Environmental or stress-specific gene regulation affects the detection rates and

approximate genomic locations of QTL (Prioul *et al.* 1999). The quality of the marker data involved in a QTL experiment plays a considerable factor in the analysis (Kearsey and Farquhar 1998). Finally, Melchinger *et al.* (1998) reported that the power of QTL detection is low for traits with a large number of minor QTL influencing the trait. These problems associated with mapping additive QTL effects are only exacerbated in QTL epistasis studies complicating interpretate of restuls from such studies.

Epistatic interactions can occur between loci that have no significant main effects. Wade (2002) suggested caution when considering the importance of significant main effects, stating, "the existence of a statistical main effect is not an indication that a gene has any effect independent of its genetic background". Holland *et al.* (1997) detected several QTL for heading date and plant height in oat that were involved in epistatic interactions. They also found epistasis among loci that were not individually significant for trait effects and concluded that all pairs of loci should be tested for epistatic interactions, not merely the significant ones, but this results in complications due to the problems of multiple testing.

Near-isogenic lines

Near-isogenic lines (NILs) are lines that are virtually genetically identical, with the exception of a relatively small proportion of their genomes. Near-isogenic lines are developed first by crossing two lines, referred to as the donor parent and the recurrent parent. The donor parent possesses a gene or chromosomal region of interest that is to be moved into the genetic background of the recurrent parent. The initial cross is followed by repeated backcrossing to the recurrent parent. Selection for the gene or genomic

region being introgressed is conducted during the backcrossing generations. During the backcrossing procedure, in the absence of selection, the rate of recurrent parent genome recovery is given by $1- (1/2)^t$ where t equals the number of backcross generations (b) plus the terminal selfing generation (i.e. $t = b + 1$) (Muehlbauer *et al.* 1988). Bartlett and Haldane (1935), Hanson (1959), Stam and Zeven (1981), and Muehlbauer *et al*. (1988) have all developed formulas to compute expected lengths of donor chromosomal segments under several types of selection.

Near-isogenic lines result from applied backcrossing breeding programs when a single gene of interest is transferred, e.g. disease resistance genes, into the background of elite cultivars. Bernacchi *et al.* (1998) developed NILs to improve cultivars of tomato through the introgression of genes from wild donor species. Storlie *et al.* (1998) used NILs to locate genomic regions associated with cold hardiness in wheat (*Triticum aestivum* L.). Tunistra *et al.* (1998) tested potential quantitative trait loci for drought tolerance in sorghum [*Sorghum bicolor* (L.) Moench] near-isogenic lines. Also, Shen *et al.* (2001) successfully introgressed QTL for root-depth into a rice cultivar. Essenberg *et al.* (2002) developed and tested NILs of cotton (*Gossypium hirsutum)* with different single resistance genes to bacterial blight (*Xanthomonas campestris* pv. *malvacearum*). As can be seen from these examples, NILs are often developed to improve a given cultivar for one additional trait of interest and then released as a new-and-improved variety.

Single near-isogenic lines are often developed to study major gene effects on various traits. Boiteux et al. (1995) listed two major advantages of NIL analysis: the minimization of epistatic effects between the gene of interest and other genes, as well as

the emphasis of major single-gene differences between lines. Kaeppler (1997) reported that, while less powerful than recombinant-inbred lines for QTL detection, NILs allow for the study of specific chromosomal regions. NILs are also a good source for map-based gene cloning and high resolution genetic mapping. Testing NILs in designed experiments allows for significant differences, or genetic effects, to be associated with specific genomic regions. Because all the entries in an experiment involving NILs are genetically similar, other than the region of interest, the background genomic influence is reduced. Despite the large amount of effort required for development, NILs present great power to precisely determine genomic regions with statistically significant effects (Stuber *et al*. 1999).

Initial studies involving the use of near-isogenic lines for the detection of epistasis began several decades ago. Fasoulas and Allard (1962) developed isogenic lines of barley (Hordeum vulgare) differing only in two chromosomal segments, and they reported that, while 65% of the overall genetic variance was attributed to additive variation, 32% was attributed to epistatic effects between those two segments. Eshed and Zamir (1996) developed double-introgression, near-isogenic lines of tomato to test for epistatic interactions among the introgressions as well as between the introgressions and background alleles. They reported that 20-42% of the 45 dichromosome segment combinations exhibited epistasis for five yield-associated traits and determined that the epistasis was less-than-additive. They also reported higher-than-expected epistatic interactions between segments that independently had no effect. This observation indicated that the number of QTL might be smaller than previously identified, and that epistatic interactions needed to be tested between all segments, not merely the siginficant

ones. It seems as if near-isogenic lines can be successful in reducing some of the background genomic issues associated with epistasis detection.

Objectives of this study

Fenster *et al.* (1997) suggested that a potential solution to measuring epistasis would be to construct specific gene combinations and test for their effects on fitness. Tanksley (1993) and Kaeppler (1997) suggested crossing NILs, with different introgressed regions, to study epistasis under uniform genetic backgrounds. This experimental design could be accomplished through the creation and testing of double-introgression near-isogenic lines (dNILs). Using dNILs will allow for greater genetic uniformity among the lines being tested and will improve effect estimates. Also, use of double-introgression NILs will help to reduce the confounding effects from the background genome and thus improve the potential detection of epistatic effects.

For our study, 127 double-introgressed, near-isogenic lines were developed by using marker-assisted selection to identify plants with the desired genotype derived from crosses between 16 NILs with different introgressions. All lines had been developed from B73 (recurrent) and Tx303 (donor) parentage. The dNILs were assigned to eight sets for replicated field experimentation. For the testcross field experiments, all double-NILs, all single NILs, B73, and Tx303 were testcrossed to Mo17. Each set included 16-18 dNILs, the single NIL parent lines that were used to generate the dNILs, B73 and Tx303. The sets were evaluated, as either testcross material or as inbred lines *per se*, under replicated lattice designs in eight North Carolina environments over two years. Questions we would like to answer with this study include **1)** Is there sufficient statistical

evidence to detect epistatic interactions between the introgressed regions for each of the agronomic traits tested (flowering time, plant and ear height, root and stalk lodging, grain yield and moisture, and seed protein and oil content)? **2)** How frequent are epistatic interactions for various traits? **3)** What are the magnitude of epistatic effects compared to the QTL main effects? **4)** Are interactions detected in inbreds related to interactions detected in hybrids?

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II. Additive genetic effects of single chromosomal segment introgressions and epistatic effects of paired introgressions on quantitative traits in maize (*Zea mays* L.): Inbred lines phenotypes

J.A. Tarter[*] and J.B. Holland[†]

[*] Department of Crop Science, North Carolina State Univ., Raleigh, NC, 27695-7620

[†] USDA-ARS, Plant Science Research Unit, Department of Crop Science, North Carolina State Univ., Raleigh, NC 27695-7620

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ABSTRACT

The detection of epistasis is often performed in conjunction with quantitative trait loci (QTL) mapping, but this results in confounding the epistatic effects with background genome segregation and QTL-by-background genome interactions. To improve the precision of estimating QTL epistatic effects, 16 chromosomal segments from Tx303 were introgressed into inbred line B73 and developed into near-isogenic lines (NILs) and then crossed in all pairwise combinations. Marker-assisted selection combined with self-fertilization was used to develop double-introgression NILs homozygous for specific pairs of introgressed segments. The resulting 127 dNILs, their 16 single-introgression parental NILs, B73, and Tx303 were evaluated in replicated field trials to measure additive and epistatic effects of the introgressed chromosomal segments. Across traits tested, between 2% and 40% of the tests involving single NILs demonstrated significant additive effects. Significant epistatic effects were detected for 2% to 27% of the dNILs. Overall the frequency and magnitude of additive effects were slightly higher than those of epistatic effects. Across traits between 16% and 80% of the significant epistatic interactions occurred between two introgressed segments that did not demonstrate singificant additive effects independently. These results suggest that predictions from marker-assisted selection of pyramided gene complexes based solely on additive effects might be biased.

INTRODUCTION

Epistasis is a form of non-additive gene action that results in a phenotype that cannot be predicted based on knowledge of the parental phenotypes. Epistatic effects can contribute to additive and dominance variances, breeding values (Cheverud and Routman 1995), and heterosis (Holland 2001; Yu *et al.* 1997). The confounding of epistatic and single-locus main effects with the additive genetic variance makes experimental estimation of epistatic effects and variances difficult (Cheverud and Routman 1995; Hallauer and Miranda 1981; Holland 2001). The limited reports of epistasis in the literature reflect a minimized importance of epistasis in breeding and genetics (Cheverud and Routman 1995). However, Routman and Cheverud (1997) suggested that epistasis (both in the positive and negative directions) can play a role in breeding populations even if the net effects are not observed. Reports of significant epistatic effects in biometric studies for numerous species have been reported, including in maize (Lamkey *et al.* 1995; Stuber and Moll 1971; Wolf and Hallauer 1997), hybrid willow (*Salix* sp.) (Fritz *et al.* 2003), pearl millet (*Pennisetum glaucum* (L.) R.Br.) (Joshi and Ugale 2002), and soybean (*Glycine max* L.) (Fasoula *et al.* 1995; Saidon *et al.* 1989).

Quantitative trait locus (QTL) mapping has often been used to test for epistasis. But, numerous problems hinder estimation of QTL main effects, and these problems are exacerabted for QTL-by-QTL epistasis. Most of these problems are caused by limited sample sizes of mapping populations (Beavis 1994; Melchinger *et al.* 1988). Limited sample sizes of QTL mapping populations result in high Type II error rates, the overestimation of QTL main effects (Beavis 1994; Lande and Thompson 1990), and a

lack of congruency between QTL studies with different samples of the same or different reference populations (Beavis 1994; Melchinger *et al.* 1988).

QTL×QTL interactions as well as QTL×background genome interactions also often complicate QTL analyses. Such interactions have been reported in maize (Doebley *et al.* 1995), rice (*Oriza sativa*) (Wu *et al.* 2000), tomato (*Lycopersicon esculentum* L.) (Monforte *et al.* 2001) and sunflower (*Helianthus spp.*) (Kim and Rieseberg 2001). Holland *et al.* (1997) found epistasis in oat (*Avena sativa)* among loci that were not individually significant for trait effects and concluded that all pairs of loci should be tested for epistatic interactions, not merely the significant ones.

Near-isogenic lines (NILs) may ameliorate some of the problems associated with QTL mapping in typical mapping population structures. The nearly uniform genomes of NILs help reduce the confounding effect of segregating backgrounds in the tested lines (Fasoulas and Allard 1962). NILs also allow for the pyramiding of genetic combinations through the crossing of differing NILs (Fenster *et al.* 1997). Studies have been performed using NILs to isolate epistatic effects. Fasoulas and Allard (1962) developed double-introgression lines of barley (*Hordeum vulgare*) with pairs of introgressions and detected epistatic variation. Eshed and Zamir (1996) developed double-introgression near-isogenic lines of tomato to test for epistatic interactions among the introgressions as well as with background alleles.

In this study, double-introgression NILs (dNILs) were compared to their inbred single-introgression parents and B73 to detect additive and additive×additive effects expressed in inbred lines. The NILs and double-introgression NILs were developed through the use of marker-assisted selection. Questions we would like to answer with

this study include 1) Is there sufficient statistical evidence to show the presence of epistatic interactions between the introgressed segments? 2) How frequent are epistatic interactions for various traits including flowering time, ear and plant height, and grain yield characters? and 3) What are the magnitudes of QTL epistatic effects compared to the main effects?

MATERIALS AND METHODS

Double-introgression near-isogenic line development: Single introgression near-isogenic lines were developed by C.W. Stuber through the introgression of Tx303 segments into B73 background using three backcross generations combined with marker-assisted selection (Hostert 2002). Sixteen single introgression NILs from the original set, with Tx303 introgressions distributed throughout the genome and minimal non-target introgressions, were selected as the starting material for this study (Table 1).

Selected NILs were crossed in all pairwise combinations in the summer of 2000. The F_1 plants were self-fertilized to generate segregating families. Marker-assisted selection (MAS) was implemented in the F_2 populations in the summer of 2001. For each cross population in the F_2 and subsequent generations, eleven or twelve individual plants were genotyped at two to six SSR loci, identified by Hostert (2002) and Szalma *et al.* (manuscript in preparation), flanking the RFLP loci to detect the presence and confirm the size of each introgression (Table 1). DNA isolation was performed on individual plants using the methods described by Riede and Anderson (1996) or Saghai-Maroof *et al.* (1994). SSR primers from Research Genetics (Huntsville, AL), Gibco BRL Life Technologies (Rockville, MD), or Genosys (Beverly, MA) were diluted to a final concentration of 5μM. PCR reactions were performed as described by Senior *et al.* (1998) and bands were separated by electrophoresis on 4% agarose gels prepared using Agarose SFR (Amersco, Solon, Ohio) and 1X TBE (Tris-Boric Acid-EDTA). DNAs were electrophoresed for approximately three h at 95 - 100V in 1X TBE buffer and PCR products were scored manually.

Individuals identified as homozygous for the Tx303 donor allele for at least one of the introgressed sites and as not homozygous for the B73 allele at the second locus were selected in the F_2 generation. A second round of marker-assisted selection was performed in the F_3 generation in the winter of 2001 and individual plants homozygous for Tx303 at both introgressions were selected (Figure 1). Additional generations of self-fertilization and MAS were performed in some cross-populations, as needed, in order to identify all desired genetic combinations. One hundred-eleven dNILs (87%) were identified in the $F_{3:4}$ generation while one additional generation of self-fertilization was necessary for 15 additional lines (12%) to be identified in the $F_{4:5}$ generation. One remaining line required four generations of self-fertilization to the $F_{5:6}$ stage to be homozygous at both target regions. The final set of dNILs included 127 independently derived double-introgression NILs representing 114 of the 120 possible pairwise introgression combinations.

Experimental design for field evaluation: Each dNIL was assigned to one of eight sets for field experiments. Each set included 15-18 double-introgression NILs, 6-8 single introgression NILs that were parents of all of the dNILs in the same set, B73, and Tx303. B73 was included between one and three times in each set as well as was used to replace entries that did not have sufficient seed supply. In 2003 and 2004, inbred trials were performed using a lattice design appropriate for each set's treatment number with two replications at three locations (Clayton, NC, Plymouth, NC, and Lewiston, NC). Only plant and ear height data were recorded at Lewiston in 2003 and at Plymouth in 2004 due

to poor germination caused by seedling rot in Lewiston and an overgrowth of weeds and intense crop-weed competition in Plymouth.

At all locations, a plot consisted of one 4.86-m row sown with 22 seeds, with a 1-m length alley at the end of each plot. In Plymouth and Clayton, rows were spaced 0.97 m, resulting in a population density of approximately 62000 plants ha^{-1} within the planted plot area. In Lewiston, inter-row spacing was 0.91 m, and population density was 66000 plants ha^{-1}.

The number of plants, mean plant height (measured from the ground to the terminal node), and mean ear height (height to node of topmost ear) were recorded for each plot at all six environments. Days to anthesis and silking were recorded only at Clayton in both years. Anthesis date was the date on which at least 50% of the plants in the plot were shedding at least 50% of the available pollen. Silk emergence date was the date on which 50% of the plants in the plot were displaying visible silks. At maturity, plots were hand-harvested. Total grain yield per plot was recorded as well as grain moisture, 100 kernel weight, protein content and oil content.

Two methods for analyzing grain protein and oil content were used for this experiment. In 2003, a sample of approximately 100g was collected from the open-pollinated seed from each plot and sent to Dr. Linda Pollak at Iowa State University for analysis. A Perstorp 6500 NIR machine (FOSS North America) was used to analyze 240ml of whole grain for protein and oil content. In 2004, grain samples were collected from open-pollinated seed from each plot and then ground to a fine particle size using a Romer Series II® Mill (Romer Labs Inc., Union, MO). Samples were analyzed using a PERCON 8620 NIR machine (Perten Instruments AB, Huddinge, Sweden) for protein

and oil content. Approximately 11% of the samples were analyzed with a Model FP-428 Nitrogen and Protein Determinator (LECO Corp. St. Joseph, MI) and with a MARAN pulsed NMR (nuclear magnetic resonance) machine (Resonance Instruments Ltd., Oxforshire, United Kingdom) to develop the NIR calibration equations for the 2004 analysis.

Statistical analysis: Data were analyzed using Proc MIXED in SAS version 8.2 (SAS Institute 2000). Each set was first analyzed separately. Entry was considered a fixed factor, whereas all other factors (replication and incomplete block within replication) were treated as random.

Obvious spatial trends within locations were observed in the data from both years. A variety of spatial analyses was performed on the data from within each environment. Models with up to fourth-order polynomial effects of rows and columns in the field layout were tested. Trend effects were maintained in the model if significant at $P < 0.01$ (Brownie *et al.* 1993). Proc MIXED was used to compare the following models within each environment: a model including complete and incomplete block effects, models with row and column trend effects selected as described, a model with correlated errors, and a model with both trend effects and correlated errors (Brownie *et al.* 1993). Within each environment, the model that minimized Akaike's Information Criterion (Lynch and Walsh 1997) and included only significant effects at $P < 0.01$ was chosen. After correcting for spatial trends, adjusted entry means for the lines were combined from all evaluation environments within each set and analyzed using Proc MIXED. Entry was

considered a fixed factor, whereas all other factors (environment and entry × environment) were treated as random.

Additive effects of introgressions *i* and *j* were estimated as :

$$\hat{a}_i = \bar{Y}_{NILi} - \bar{Y}_{B73}$$
$$\hat{a}_j = \bar{Y}_{NILj} - \bar{Y}_{B73}$$

where $\bar{Y}_{NILi}$ is the mean of the NIL carrying introgression *i* and $\bar{Y}_{B73}$ is the mean of inbred B73. Since each introgression region was represented by a NIL that was included in multiple sets, we report the proportion of additive effects that were significant among all tests (a total of 58 NIL-set combinations). Epistatic effects between introgressions *i* and *j* were estimated as :

$$aa_{i\times j} = \frac{\left(\bar{Y}_{dNILi\times j} + \bar{Y}_{B73}\right)}{2} - \frac{\left(\bar{Y}_{NILi} + \bar{Y}_{NILj}\right)}{2}$$

where $\bar{Y}_{dNILi\times j}$ is the mean of the double-introgression NIL carrying segments *i* and *j*. For nine pairs of introgressions, multiple dNILs representing each combination were independently derived. For such cases, the average of all lines representing that combination was used in the epistasis estimates. Additive and epistatic effects were estimated using "estimate" statements in Proc MIXED and were considered significant at $P < 0.05$ because only pre-planned comparisons were made.

Three types of epistasis, synergistic, antagonistic, and non-canceling, were observed in the dNILs and classified according to the performance of the parental line

from the appropriate set. Synergistic epistasis occurs when epistatic effects occur in the same direction as the additive effects (Crow 1970). Antagonistic epistasis occurs when the epistatic effects occur in the opposite direction of the two additive effects (Crow and Kimura 1970). We suggest the term "non-canceling epistasis" to explain the type of gene action observed when the additive gene effects are in opposite directions and yet significant epistasis is detected, i.e., when the additive effects fail to cancel each other out. However, depending on how the gene effects are labeled, any example of non-canceling epistasis can be reclassified as either synergistic or antagonistic epistasis.

Additional population estimates were calculated for genotype-by-environment (G×E) interactions and phenotypic correlations. In order to test for genotype-by-environment interactions, additive and epistatic effects from the adjusted spatial trend analyses from all sets were combined. We reported the proportion of additive and epistatic effects that were significant among all tests (a range of 102-347 tests for additive effects and 206-763 tests for epistatic effects). Finally, to calculate phenotypic correlations between traits, adjusted entry means for each line except Tx303 within each set were subtracted from the appropriate overall set mean. These resulting effect estimates were combined and used to estimate phenotypic correlations using PROC CORR in SAS.

RESULTS

Phenotypic trait correlations: Twenty of 36 (56%) pairs of traits were significantly correlated (Table 6). Days to anthesis and silk were highly correlated (r = 0.71), as were ear and plant height (r = 0.81). A strong positive correlation between protein and oil content also existed (r = 0.73). All other correlations had absolute values less than 0.50 (Table 6).

Significant additive and epistatic effects: Significant additive effects were detected in the single introgression NILs and significant epistatic effects were detected in the double-introgressions for all nine traits tested (Table 2). Among traits, from 2% to 40% of the tests for additive effects of chromosomal introgressions were significant ($P < 0.05$). Additive QTL effects were detected least often for flowering time, with only two and three percent of tests for additive effects significant for days to anthesis and days to silk, respectively. However, 40% of the tests involving single chromosomal regions demonstrated significant additive effects for plant height. Significant additive effects for total grain yield were detected in 22% of the tests involving single introgressed regions. For five of the nine traits (56%) traits, both significant positive and negative effects were detected for Tx303 introgressions throughout the genome.

Due in part to the variability associated with field testing, the significant effects detected for some introgressions were found in only a subset of the experiments in which the NIL carrying that introgression was included (Tables 3). Averaging across introgressions and traits, 26 of the 44 (59%) examples of significant additive gene action effects occurred in less than 50% of the sets in which they were tested. Only nine of the

44 (20%) examples of additive gene action were statistically significant in all sets in which they could be tested. Plant height QTL appeared the most stable across sets, with six of the eight (75%) chromosomal regions being detected in at least 75% of sets in the field. Protein content QTL were among the least stable with all of the eight QTL being detected in less than 50% of the sets in which they could be tested.

Among traits, from 2% to 27% of the tests involving introgression pairs exhibited significant epistasis (Table 2). As with additive effects, epistasis was least common for days to anthesis and days to silk (four and two percent, respectively). Also following the pattern of additive effects, the greatest percentage of epistatic effects were detected for ear and plant height (21% and 27%, respectively). Epistatic effects in both the positive and negative directions were detected for seven of the nine traits (78%).

Genotype-by-environment interaction: The analysis within environments led to a greater detection of significant additive and epistatic effects than the across-environments analysis. All traits, except ear and plant height, demonstrated significant environment-specific genetic effects more frequently than significant effects averaged across environments. Across traits (except ear and plant height), there was between a zero and 600% increase in significant additive effects when analyzing within environments. For epistatic effects across traits, the increase of significant effects was between 0% and 500%.

Effects of specific chromosomal segments: Fourteen of the 16 chromosomal regions demonstrated significant additive effects for at least one of the traits tested (Table 3).

The chromosomal regions located on 1L and 4S were not involved in any significant additive effects for any of the traits tested. However, the chromosomal region on 6C was involved in significant additive effects for ear and plant height, grain yield, kernel weight, and protein and oil content. The introgression located on 10S was involved in significant additive effects for days to silk, grain yield and moisture, kernel weight, and protein content.

All 16 chromosomal regions were involved in at least one significant epistatic interaction for at least one of the traits tested (Table 4). Individual introgressions were involved in significant epistatic interactions for between 33% and 89% of the traits. The introgression on 7L was the only chromosomal region involved in significant epistatic effects for less than 40% of the traits tested. The chromosomal regions on 4S and 7S were involved significant epistatic effects for 89% of the traits tested. The only trait for which neither the 4S or 7S introgressions were involved in significant epistatic interactions for was days to anthesis.

Types of epistasis: All three types of epistasis were exhibited by the dNIL inbred lines in these experiments (Table 5). Antagonistic epistasis was, by far, the most common of the three types. Between 20% and 100% of the epistasis detected was considered antagonistic. Antagonistic epistasis was the only type of epistasis detected for all traits. Non-canceling epistasis was the second most common form of epistasis detected and it ranged between 0% and 80% of the epistasis across traits. The only trait for which non-canceling epistasis was not detected was oil content. Synergistic epistasis was detected at least once for only four traits (days to silk, ear height, plant height, and grain yield).

Synergistic epistasis never accounted for more than 20% of the epistatic interactions detected for any one trait.

This experiment permitted determining the relative frequency with which epistatic effects were caused by introgressions that individually represented 0, 1, or 2 significant main effects (Table 5). Across traits, eleven percent of the epistatic interactions involved loci that both had significant additive effects. Among traits, 0% to 58% of epistatic pairs involved one introgression with a significant additive effect and one with no detected additive effect. From 16% to 83% of the significant epistatic effects detected were between two chromosomal regions with no individual significant additive effects. For the simpler traits of plant and ear height, most (63% and 68%, respectively) significant epistatic effects involved only one region with a significant main effect. For grain yield, 40% of the significant epistatic effects involved two regions with significant additive effects, 20% involved one region with significant additive effects, and 40% involved two regions that had no significant additive effects. For grain moisture, five percent of the significant epistatic effects involved two regions with significant additive effects, 16% involved one region with a significant additive effect, and 79% involved two regions that both had no significant additive effects.

DISCUSSION

Technical limitations of this study: Our experiment has several potential shortcomings that might bias the estimates of additive and epistatic effects. The first problem is the possibility of the loss of a portion of the introgressed segment due to recombination during the double-introgression line development because selection was conducted only on flanking markers. Failure to retain the introgressed segment would result in a biased estimate of the epistatic effect and invalid significant tests for epistasis. If both introgressions have positive additive effects and one or both are lost during dNIL development, a negative (antagonistic) epistatic effect could be detected, although epistasis was not the true cause of the dNIL's deviation from expectation based on sums of additive effects. Similarly, non-canceling epistasis could be detected incorrectly due simply to the loss of the positive or negative QTL region. Importantly, synergistic epistasis would not result from loss of either of the QTL region, regardless of the significance of their effects. Thus, our experiment may have a systematic bias toward detecting antagonistic epistasis.

Additional genotyping is currently being performed on the entire span of the introgressed segments in order to test this theory. After the genotypic verification of all dNILs is complete, we can remove any dNILs that have lost a significant portion of one of the introgression regions from consideration. After this is completed, any systematic bias toward detecting antagonistic epistasis should be eliminated.

A second potential pitfall is the background variability remaining in the single-introgression NILs used to develop the dNILs. According to Hostert (2002), an average of less than four percent of the background genome of the parental NILs is composed of

non-target introgressions. These non-target introgressions might be responsible for confounding of some background genetic effects with the effects of the introgressions. Furthermore, these non-target introgressions were not monitored during dNIL development. Therefore both additive and epistatic interactions could be confounded with the effects of the genetic background.

Trait correlations: In a few cases, significant additive effects for multiple traits were exhibited by the same chromosomal segment. Despite the high phenotypic correlation between days to anthesis and days to silk, neither of the significant chromosomal regions associated with additivity for either trait were in common between the two traits (Table 3). This lack of a correlation of genetic effects might be affected by the low level of independent significant additive effects detected. However, six of the seven (86%) regions associated with significant additive effects for ear height demonstrated significant additive effects for plant height as well. Four of the five (80%) chromosomal regions associated with additivity for kernel weight were in common with those associated with grain yield ($r = 0.50$, Tables 3 and 6). On the other hand, significant additive effects for grain yield were also associated with three other regions that did not affect kernel weight. The additional regions of significance indicated that genes associated with grain yield include more than those merely associated with kernel weight. Only two of the chromosomal regions that demonstrated significant additive effects for either protein or oil content ($r = 0.73$) were in common for both traits (Tables 3 and 6). One of the introgressed segments (6C) demonstrated significant additive effects in the same direction for both traits. However, the other introgression (9S) exhibited a positive

additive effect on protein content while exhibiting a negative additive effect on oil content.

When considering chromosomal regions that demonstrated significant epistatic effects, the trends were quite similar to those of the additive effects (Table 4). Only two pairs of introgressions associated with significant epistatic effects were in common between days to anthesis and days to silk. One pair demonstrated a positive effect on both days to anthesis and days to silk, while the other demonstrated a negative effect on both traits. When considering ear and plant height epistasis, twelve pairs of introgressions displaying significant epistatic effects were in common for both traits. Positive and negative epistatic effects were noted for both traits. But the directions of the epistatic effects for each pair were the same in both traits. Only two pairs of introgressions were in common for significant epistatic effects between grain yield and kernel weight. However the direction of the effects on the traits differed. In both cases, grain yield demonstrated a negative epistatic effect and kernel weight demonstrated a positive epistatic effect. One possible explanation would be that a reduction in yields in this dNIL were caused by incomplete seed set. However, the kernels that did exist developed into larger kernels because they were not constrained by a full seed set, thus resulting in a larger kernel weight. Only two pairs of chromosomal regions were associated with significant epistatic effects for grain yield and moisture. Both pairs led to an increase in grain yield and a reduction in grain moisture. Finally, zero pairs of introgressed segments that demonstrated significant epistatic effects were shared between both protein and oil content.

Relationship between additivity and epistasis: For most traits, significant additive effects occurred at approximately the same frequencies as significant epistatic effects. The mean of the absolute value of the effects was slightly larger for the additive effects for some of the traits. But, the rates of detection for the two types of genetic effects differed by more than five percent for only three of the traits tested (plant height, grain yield, and protein content). In this study, we used a significance threshold of $\alpha = 0.05$, resulting in a five percent chance of making a Type I error and declaring a false positive. The percentage of significant additive and epistatic effects for both days to tassel and silk was lower than five percent. At such a low frequency, those tests could have been declared significant by chance alone, suggesting that no QTL for flowering time were segregating among the NILs.

The chromosomal segment located on 4S was involved in the largest percentage (89% of the traits) of epistatic interactions but had no significant additive effect for any trait. A similar trend was exhibited by the introgression located on 1L. That chromosomal region was involved in significant epistatic interactions for 78% of the traits tested, however it exhibited no significant additive effects. In contrast, the chromosomal region of 6C was involved in significant epistatic effects for 85% of the traits (the second highest percentage) as well as being involved in significant additive effects for 57% of the traits (the highest percentage). The variability across the chromosomal segments' genetic actions implies that some of these tested regions might be involved in complex pathway systems and thus more sensitive to other genetic regions than those outside of complex pathways.

Influence of genotype-by-environment interactions: For all traits, except ear and plant height, the detection rates of significant additive and epistatic effects were higher within environments compared to the same effects averaged across environments. This indicates that for traits other than ear and plant height the environment-specific gene effects were more common than environmentally stable gene effects. This was true for both additive and epistatic gene effects.

Conclusions: Significant additive and epistatic interactions were detected among single introgression and double-introgression chromosomal segments in the near-isogenic lines. Overall, significant additive effects were detected at a greater frequency than significant epistatic effects, although the differences were small in most cases. However, absolute values of the epistatic effects were lower than those of the additive effects. Eshed and Zamir (1996) reported similar findings when testing double-introgression NILs of tomato. They found significant epistatic interactions for between 20 and 42% of the pairs of introgressions for five different fruiting traits, and the epistasis was always less-than-additive for QTL affecting traits in the same direction. To our knowledge, these are the only two epistasis studies involving lines specifically developed to test introgression interactions under a uniform background genome. Both studies reported the presence of signficant epistatic interactions for numerous traits and that epistasis was usually less-than-additive, or antagonostic, in nature. These results lead one to be concerned about the overestimation of performance predictions based solely on the pyramiding of additive effects of chromosomal regions of interest.

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TABLE 1. Summary of introgressed regions, their RFLP probes and chromosomal bins, and SSR loci and chromosomal bins used in the marker-assisted selection program.

Genomic Location	Left Flanking SSR	SSR Bin[a]	RFLP Clone	Right Flanking SSR	SSR Bin
1L	bnlg1720	1.10	umc107	umc1862	1.11
2L	bnlg1045	2.07	umc122	umc1560	2.07
3S	phi104127	3.01	umc32a		
4S	umc1550	4.03	bnlg5.46		
4L	bnlg1265	4.05	bnlg8.23	umc2011	4.10
5S			umc147a	bnlg565	5.02
5L			umc68	bnlg1306	5.07
6C	umc1887	6.04	umc21	bnlg1922	6.05
6S	phi126	6.00	umc85	bnlg1538	6.01
7S			umc116	bnlg434	7.03
7L	bnlg469	7.05	umc168	phi116	7.06
8S	umc2146	8.03	umc32b	umc1130	8.05
9S	bnlg1724	9.01	umc113	umc1771	9.04
9L			nlg14.28	bnlg1525	9.07
10S	phi050	10.03	umc155	umc1589	10.04
10L			umc44a	umc1045	10.06

[a] Bin location based on recombination frequency. Bin positions are defined by core RFLP markers (Davis *et al.* 1999) and span approximately 20cM.

TABLE 2. Range, mean of absolute values, and proportion of significant additive and epistatic effects identified in single-introgression and double-introgression near-isogenic inbred lines, *per se*, evaluated in two to six North Carolina environments.

	Days to anthesis[a]	Days to silk[a]	Ear height	Plant height	Grain yield	Grain moisture	Kernel weight	Protein content[b]	Oil content[b]
Additive Effects									
	d	d	m	m	g plot^{-1}	%	g	%	%
Range	2.4	2.4,3.0	-0.11,0.17	-0.20,0.23	-593,-329	3.1,6.4	-0.06,0.03	-1.9,1.8	-0.5,0.8
Mean of absolute values	2.4	2.7	0.10	0.14	451	4.9	0.03	1.5	0.6
Introgressed chromosome segments with significant additive effects									
	%	%	%	%	%	%	%	%	%
Additive effects averaged across environments [58 tests]									
	2	3	19	40	22	16	12	17	10
Environment-specific additive effects [102-347 tests]									
	12	16	18	29	25	26	18	24	10

TABLE 2. Continued.

	Days to anthesis[a]	Days to silk[a]	Ear height	Plant height	Grain yield	Grain moisture	Kernel weight	Protein content[b]	Oil content[b]
Epistatic Effects									
	d	d	m	m	g plot^{-1}	%	g	%	%
Range	-2.2,2.6	-1.9,2.5	-0.08,0.22	-0.12,0.23	-497,356	-4.4,4.8	0.01,0.04	0.9,2.0	-0.9,0.5
Mean of absolute values	2.1	1.9	0.07	0.09	326	3.2	0.02	1.5	0.5
Introgressed segments demonstrating significant epistatic effects									
	%	%	%	%	%	%	%	%	%
Epistatic effects averaged across environments [114 tests]									
	4	2	21	27	13	17	10	7	9
Environment-specific additive effects [206-763 tests]									
	13	10	14	21	16	29	14	11	18
Mean F-values for genotypes averaged in the combined analysis across sets									
	6.36	5.10	8.36	9.44	3.69	7.05	7.28	4.43	2.72

[a] Days to anthesis and silk are recorded at Clayton only.

[b] Protein and oil data reported from 2003 only.

TABLE 3. Summary of introgressed regions, their RFLP probes, and percentage of sets containing the sNIL carrying the introgression in which the additive effect was significant for each trait, base on inbred NILs *per se* evaluated in 6 North Carolina environments.

Genomic Location	RFLP Clone	Days to Anthesis	Days to Silk	Ear Height	Plant Height	Grain Yield	Grain Moisture	Kernel Weight	Protein Content	Oil Content
1L	umc107									
2L	umc122				75	100			50	
3S	umc32a					67	100		33	
4S	bnlg5.46									
4L	bnlg8.23			25	100					25
5S	umc147a	25			25	25		25		
5L	umc68					67			33	
6C	umc21			33	100	67		33	33	100
6S	umc85			66	100				33	33
7S	umc116			25	100				50	
7L	umc168			25	25		25			
8S	umc32b			100	100		25	75		
9S	umc113								25	25
9L	bnlg14.28			33						
10S	umc155		50			25	100	25	33	

TABLE 3. Continued.

10L	umc44a	33	33

TABLE 4. Summary of introgressed regions, their RFLP probes, and percentage of dNILs carrying an introgression in which significant epistatic effects were detected for each trait, based on inbred NILs *per se* evaluated in 6 North Carolina environments.

Genomic Location	RFLP Clone	Days to Anthesis	Days to Silk	Ear Height	Plant Height	Grain Yield	Grain Moisture	Kernel Weight	Protein Content	Oil Content
1L	umc107		14	21	21	7		7	7	7
2L	umc122	13	8	27	40	20	13	20		
3S	umc32a			7	21	21	64			7
4S	bnlg5.46		14	21	29	14	14	7	21	7
4L	bnlg8.23		8	31	46			8		31
5S	umc147a	14		36	14	29	7	7		21
5L	umc68	7	7		47	20	20			
6C	umc21	14		43	36	29	14	7		36
6S	umc85	13		33	40	7	13		13	7
7S	umc116		7	33	33	7	13	13	13	13
7L	umc168				7		20		13	
8S	umc32b			33	33	20	27	47	13	
9S	umc113		7	21	14	7		7	7	
9L	bnlg14.28	7	7	7	14		21	7	7	
10S	umc155		7	14	14		7	7	7	7

TABLE 4. Continued.

10L	umc44a	8	8	23	31	31	15	8

TABLE 5. Summary of types of epistasis exhibited by double-introgression NILs *per se* with significant epistatic effects and significant parental additive effects combined over all sets in 6 North Carolina environments.

	Days to anthesis	Days to silk	Ear height	Plant height	Grain yield	Grain moisture	Kernel weight	Protein content	Oil content
	%	%	%	%	%	%	%	%	%
Synergistic epistasis	0	17	8	7	7	0	0	0	0
Antagonistic epistasis	20	50	58	65	80	84	73	75	100
Non-canceling epistasis	80	33	33	29	13	16	27	25	0

Proportion of significant epistatic effects involving 0, 1, or 2 chromosomal segments with significant additive effects

No. of segments with significant additive effects	%	%	%	%	%	%	%	%	%
0 parents	80	83	33	16	40	79	27	50	44
1 parent	20	17	63	68	20	16	33	50	44
2 parents	0	0	4	16	40	5	0	0	11

TABLE 6. Correlation coefficients between agronomic traits calculated from double-introgression NIL trait mean deviations.

	Days to Silk	Ear Height	Plant Height	Grain Yield	Grain Moisture	Kernel Weight	Protein Content	Oil Content
Days to Anthesis	0.71**	0.28**	0.27**	-0.35**	0.19*	0.07	0.10	-0.09
Days to Silk		0.18*	0.19*	-0.38**	0.40**	-0.10*	0.08	-0.09
Ear Height			0.81**	0.23*	0.09*	0.12	-0.08	-0.17*
Plant Height				0.04	0.19*	0.03	0.09	-0.12
Grain Yield					-0.15*	0.50**	-0.31**	0.07
Grain Moisture						-0.11	0.10	0.03
Kernel Weight							-0.21*	0.09
Protein Content								0.73**

* Indicates significant value at P ≤0.05.

** Indicates significant value at P ≤0.001.

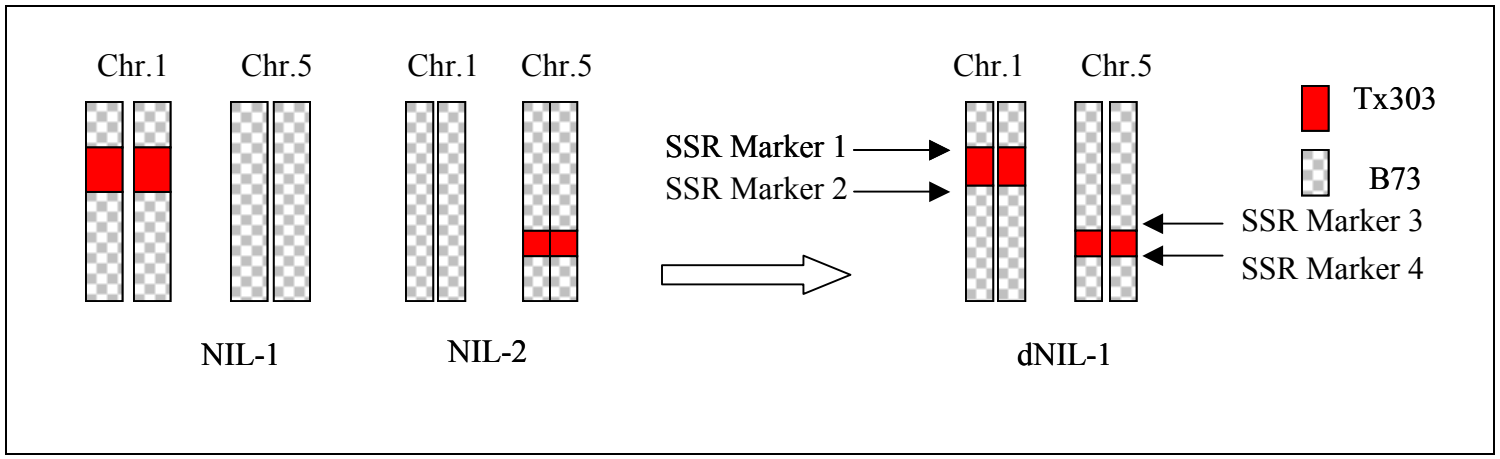

FIGURE 1. Diagram of double-introgression NIL development using MAS.

III. Additive genetic effects of single chromosomal segment introgressions and epistatic effects of paired introgressions on quantitative traits in maize (*Zea mays* L.): Testcross hybrid phenotypes

J.A. Tarter[*] and J.B. Holland[†]

[*] Department of Crop Science, North Carolina State Univ., Raleigh, NC, 27695-7620

[†] USDA-ARS, Plant Science Research Unit, Department of Crop Science, North Carolina State Univ., Raleigh, NC 27695-7620

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ABSTRACT

Epistasis effects estimated in conjunction with quantitative trait locus (QTL) mapping are often confounded by segregating background genome or QTL × background interactions. In order to reduce those effects, 16 near-isogenic lines (NILs), resulting from the introgression of chromosomal segments of Tx303 into the genetic background of inbred line B73, were crossed in all pairs. Marker-assisted selection and self-fertilization were employed to create double-introgression NILs (dNILs) homozygous for two introgressed segments. The resulting 127 dNILs, their 16 parental, single-introgression NILs, and inbred lines B73 and Tx303 were testcrossed to Mo17 and evaluated in field trials to measure the effects of introgressions singly (additive effects) and in pairs (epistatic effects). Across traits tested, between 2% and 28% of the tests involving single NILs demonstrated significant additive effects. Significant epistatic effects were identified in 2% to 19% of the dNILs. Generally, the frequency and magnitude of epistatic interactions were less than those of additive effects. Across traits, between 0% and 100% of the significant epistatic interactions involved chromosomal segments that did not display independent significant additive effects. These results suggest that epistatic interactions can affect predictions of phenotypic performance based solely on additive effects detected either in inbred lines or hybrid combinations.

INTRODUCTION

Epistasis is a form of non-additive gene action that is characterized by a phenotypic value not predicted by the sum of the individual locus values. Epistatic variance contributes to the estimates of additive and dominance variance, and thus breeding values, in a given population (Cheverud and Routman 1995). Epistasis can also cause additive variance to increase more than expected under inbreeding (Cheverud and Routman 1996) and contribute to heterosis (Holland 2001).

Difficulties in experimentally detecting and estimating epistatic effects, compared to estimates of additive gene effects, hinder our understanding of its importance in natural and breeding populations. Melchinger (1987) noted that biometrical approaches like generation means analyses measure net epistasis which may be minimized by the cancellation of positive epistatic effects by negative effects. For example, Hinze and Lamkey (2003) reported that epistasis for grain yield in maize was not as prevalent as they expected, and they attributed the failure to detect epistasis to several conditions including the cancellation of negative and positive effects. Routman and Cheverud (1997) suggested that "the failure … to find epistasis does not mean that physiological epistasis does not occur". However, significant epistatic effects have been reported in biometrical studies for a number of species for at least 50 years. Epistatic interactions have been reported in maize (Lamkey *et al.* 1995; Stuber and Moll 1971; Wolf and Hallauer 1997), hybrid willow (*Salix* sp.) (Fritz *et al.* 2003), pearl millet (*Pennisetum glaucum* (L.) R.Br.) (Joshi and Ugale 2002) and peanut (*Arachis hypogea* L.) (Upadhyaya and Nigam 1999).

As an alternative to biometrical analysis, quantitative trait loci (QTL) mapping can be used to test for epistasis. However, limited population sizes prevent accurate simultaneous estimations of multiple QTL effects. QTL studies are often conducted in populations of small sample size, resulting in high Type II error rates, overestimation of effects of those QTL that are detected, and poor predictive power of QTL models between different populations and even different samples of the same population (Beavis 1994; Melchinger *et al.* 1988).

QTL are sometimes involved in epistatic interactions with other QTL as well as QTL-background genome interactions. Such interactions have been reported in maize (Doebley *et al.* 1995), rice (*Oriza sativa*) (Wu *et al.* 2000), rapeseed (*Brassica napus* L.) (Zhao *et al.* 2005), and tomato (*Lycopersicon esculentum* L.) (Monforte *et al.* 2001). Holland *et al.* (1997) found epistasis in oat (*Avena sativa)* among loci that were not individually significant for trait effects and concluded that all pairs of loci should be tested for epistatic interactions, not merely the significant ones.

In addition to problems due to limited sample sizes, the lack of genetic uniformity among the lines being tested (Kaeppler 1997) and the confounding effects of background alleles (Fasoulas and Allard 1962) often hinder estimates of epistasis. Tanksley (1993) and Fenster *et al*. (1997) suggested that a potential solution to measuring epistasis would be to construct specific gene combinations and test their effects on fitness. This could be accomplished through the creation and testing of double-introgression near-isogenic lines (dNILs)

Initial studies involving the use of near-isogenic lines (NILs) for the detection of epistasis began several decades ago. Fasoulas and Allard (1962) developed isogenic lines

of barley (*Hordeum vulgare*) with two introgressions and detected epistatic variation between the two segments. They reported that 65% of the variation was attributed to additive variance and 32% was attributed to epistatic effects between the two segments. Eshed and Zamir (1996) developed double-introgression, near-isogenic lines of tomato to test for epistatic interactions among the introgressions as well as background alleles. They detected significant epistatic interactions for 20 – 42% of the dichromosome segment combinations when testing five yield traits. They also reported that the observed epistasis was less-than-additive and occurred between introgressions than did not possess significant additive effect independently less frequently than expected.

Epistasis can contribute to the heterosis observed in maize hybrids. Maize is a cross-pollinated species cultivated almost exclusively as F_1 hybrids resulting from crosses between pairs of unrelated inbred lines. F_1 hybrids are maximally heterozygous and maximally vigorous, whereas inbred lines of maize suffer substantial inbreeding depression (Fehr 1987). Heterosis is critical to the success of hybrid maize; however, the significant genetic gains achieved in hybrid maize yields in the past century have been mostly due to improved additive effects observed in both inbred lines and hybrids, whereas the amount of heterosis has changed little (Duvick 1999). Since epistasis can affect phenotypes of both inbreds and hybrids, attempts to measure epistasis in maize should include both.

In this study, we measured the effects of introgressing chromosomal regions singly and in pairs from inbred line Tx303 to inbred B73, as observed in hybrids created by testcrossing the resulting single-introgression and double-introgression NILs to unrelated inbred Mo17. The effects of single introgressions were considered additive

effects because the testcross phenotypes measured were considered in relation to the value of the control B73 × Mo17 testcross. Similarly, the residual genetic effects of the double-introgression NILs not accounted for by the introgression main effects were considered additive-by-additive epistatic effects. In this case, loci or locus pairs at which the Mo17 allele was completely dominant over both B73 and Tx303 alleles would not have detectable effects. However, since the testcross population is the reference population (B73 × Mo17) , the effects can be considered additive and additive×additive testcross effects (Melchinger *et al.* 1998).

Through the use of marker-assisted selection, we developed double-introgression near-isogenic lines of maize to estimate epistatic variation in several agronomic traits. Questions we would like to answer with this study include 1) Is there sufficient statistical evidence to show the presence of epistatic interactions between the introgressed segments? 2) How frequent are epistatic interactions for various traits including flowering time, ear and plant height, and grain yield characters? 3) What are the magnitudes of epistatic effects compared to the QTL main effects? and 4) Do the detection rates and estimates of additive and epistatic gene effects differ between dNIL inbred lines, *per se*, and dNIL lines in testcrosses?

MATERIALS AND METHODS

Double-introgression near-isogenic line development: The development of the double-introgression near-isogenic lines of maize involving Tx303 introgressions in a B73 background are presented in depth elsewhere (Tarter 2005). Briefly, 16 original NILs developed by C.W. Stuber (Hostert 2002) were crossed in all pairwise combinations in an attempt to introgress two different Tx303 regions into single inbred lines. Marker-assisted selection was used to select individual plants homozygous for Tx303 at both introgressed loci and self-fertilization continued until the lines were considered to be completely inbred. One hundred-eleven dNILs (87%) were identified in the $F_{3:4}$ generation while one additional generation of self-fertilization was necessary for 15 additional lines (12%) to be identified in the $F_{4:5}$ generation. The remaining line required a total of four generations of self-fertilization to the $F_{5:6}$ stage. At the completion of the line development, 114 of the 120 potential introgression combinations were identified and developed into 127 independently-derived double-introgression NILs.

Experimental design: Each dNIL, parental NIL, B73, and Tx303 were testcrossed to Mo17 to generate hybrid testcross seed in the summer of 2002. Each dNIL was assigned to one of eight sets for field experiments using a lattice design. Each testcross set included 15 to 18 double-introgression NIL testcrosses, 6 to 8 single introgression NIL testcrosses that were parents of all of the dNILs in the same set, B73 × Mo17, and Tx303 × Mo17. B73 testcross was included between one and three times in each set as well as used to replace entries that did not have sufficient seed supply for all replications. Hybrid

testcross trials were performed with two replications at four locations in 2003 and 2004 (Clayton, NC, Jackson Springs, NC, Plymouth, NC, and Lewiston, NC).

At all locations, plots were two 4.86-m rows sown with 44 seeds, with a 1-m length alley at the end of each plot. In Clayton, Plymouth, and Jackson Springs, rows were spaced 0.97 m, resulting in a population density of approximately 62,000 plants ha^{-1} within the planted plot area (excluding alleys). In Lewiston, inter-row spacing was 0.91 m, and population density was 66,000 plants ha^{-1}.

The numbers of plants, mean plant height (measured from the ground to the terminal node), and mean ear height (height to node of topmost ear) were recorded for each plot at all eight environments. Days to anthesis and silking were recorded only at Clayton in both years. Anthesis date was the date on which at least 50% of the plants in the plot were shedding at least 50% of the available pollen. Silk emergence date was the date on which 50% of the plants in the plot were displaying visible silks. In 2004, four of the eight sets in Jackson Springs had less than 30% seedling emergence due to extreme infestations of soil-borne pests. No data were recorded and these sets were not included in the final data set.

Counts were taken of root-lodged plants (leaning greater than 30° from vertical with intact stalks) and stalk-lodged plants (broken below the ear or plants with dropped ears) in all environments except Plymouth in 2003 and 2004. In 2003, Hurricane Isabel made landfall on the coast of North Carolina before harvest and resulted in a total loss of lodging and grain yield data at Plymouth. In 2004, several tropical storms passed over Plymouth during the course of the growing season resulting in substantial lodging, thus a count of number of erect plants was taken prior to harvest.

At maturity, plots were machine harvested, and grain yield and moisture content were recorded for each plot. Grain yields were adjusted to 155 g kg^{-1} moisture content. Additionally, two ears per plot were sampled for grain analysis at locations with corresponding inbred trials (Clayton and Plymouth in 2003; Clayton and Lewiston in 2004). Grain analysis included measuring 100 kernel weight, percent protein content, and percent oil content.

Grain protein and oil content analysis was performed in 2003 by sampling approximately 100g of grain from the open-pollinated seed from each plot and sent to Dr. Linda Pollak at Iowa State University for analysis. A Perstorp 6500 NIR machine (FOSS North America) was used to analyze 240 ml of whole grain for protein and oil content. In 2004, open-pollinated grain samples were collected and ground to a fine particle size using a Romer Series II® Mill (Romer Labs Inc., Union, MO). Samples were analyzed at North Carolina State University using a PERCON 8620 NIR machine (Perten Instruments AB, Huddinge, Sweden) for protein and oil content. Approximately 11% of the samples were analyzed with a Model FP-428 Nitrogen and Protein Determinator (LECO Corp. St. Joseph, MI) and with a MARAN pulsed NMR (nuclear magnetic resonance) machine (Resonance Instruments Ltd., Oxforshire, United Kingdom) to develop the NIR calibration equations for the 2004 analysis.

Statistical analysis: The percent of erect plants for each plot was calculated as the number of plants neither stalk nor root lodged, divided by the stand count. Data were analyzed using Proc MIXED in SAS version 8.2 (SAS Institute 2000). Each set was analyzed separately. Entry was considered a fixed factor, whereas all other factors

(replication and incomplete block within replication) were treated as random. Percent stand was used as a covariate for grain yield only when significant at P <0.01.

Obvious spatial trends within locations were observed in the data from both years. A variety of spatial analyses was performed on the data from within each environment. Models with up to fourth-order polynomial effects of rows and columns in the field layout were tested. Trend effects were maintained in the model if significant at P < 0.01 (Brownie *et al.* 1993). Proc MIXED was used to compare the following models within each environment: a model including complete and incomplete block effects, models with row and column trend effects selected as described, a model with correlated errors, and a model with both trend effects and correlated errors (Brownie *et al.* 1993). Within each environment, the model that minimized Akaike's Information Criterion (Lynch and Walsh 1997) and included only significant effects at P < 0.01 was chosen. After correcting for spatial trends, adjusted entry means for the lines were combined from all eight evaluation environments within each set and analyzed using Proc MIXED. Entry was considered a fixed factor, whereas all other factors (environment and entry × environment) were treated as random.

Additive effects of introgressions *i* and *j* were estimated as :

$$\hat{a}_i = \overline{Y}_{NILi} - \overline{Y}_{B73}$$
$$\hat{a}_j = \overline{Y}_{NILj} - \overline{Y}_{B73}$$

where $\overline{Y}_{NILi}$ is the mean of the NIL testcross carrying introgression *i* and $\overline{Y}_{B73}$ is the mean of B73 × Mo17. Since each introgression region was represented by a NIL that was included in multiple sets, we reported the proportion of additive effects that were significant among all tests (a total of 58 NIL-set combinations). Epistatic effects between introgressions *i* and *j* were estimated as :

$$aa_{i\times j} = \frac{\left(\overline{Y}_{dNILi\times j} + \overline{Y}_{B73}\right)}{2} - \frac{\left(\overline{Y}_{NILi} + \overline{Y}_{NILj}\right)}{2}$$

where $\overline{Y}_{dNILi\times j}$ is the mean of the dNIL carrying introgressions *i* and *j*. For nine pairs of introgressions, multiple dNILs representing each combination were independently derived. In these cases, the average of all lines representing that combination was used in the epistasis estimates. Additive and epistatic effects were estimated using "estimate" statements in Proc MIXED and were considered significant at $P < 0.05$ because only pre-planned comparisons were made.

Three types of epistasis, synergistic, antagonistic, and non-canceling, were observed in the dNILs and classified according to the performance of the parental lines from the appropriate set. Synergistic epistasis occurs when epistatic effects occur in the same direction as the additive effects (Crow 1970). Antagonistic epistasis occurs when the epistatic effects occur in the opposite direction of the two additive effects (Crow and Kimura 1970). We suggest the term "non-canceling epistasis" to explain the type of gene action observed when the additive gene effects are in opposite directions and yet significant epistasis is detected, i.e., when the additive effects fail to cancel each other out.

Additional population estimates were calculated for genotype-by-environment (G × E) interaction and phenotypic correlations. In order to test for genotype-by-environment interactions, additive and epistatic effects from the adjusted spatial trend analyses from all sets were combined. We reported the proportion of additive and epistatic effects that were significant among all tests (a range of 115-436 tests for additive effects and 209-794 tests for epistatic effects). Finally, to calculate phenotypic

correlations between traits, adjusted entry means for each line except Tx303 × Mo17 within each set were subtracted from the appropriate overall set mean. These resulting effect estimates were combined and used to estimate phenotypic correlations using PROC CORR in SAS.

RESULTS

Phenotypic trait correlations: Twenty of 45 pairs of traits were significantly correlated (Table 5). Days to anthesis and silk were highly correlated (r = 0.57), as were ear height and plant height (r = 0.71). All other correlations had absolute values less than 0.30 (Table 5).

Significant additive and epistatic effects: Significant additive and epistatic effects were detected for all 10 traits evaluated (Table 1). The percentage of tests that were significant for chromosomal introgression additive effects averaged across environments ranged from two percent to 28% among traits. Days to anthesis and silk were the traits with the fewest introgressions exhibiting additivity, two percent for each trait. Significant additive effects for ear and plant height were detected in 16% and 28% of the NIL tests, respectively. Four of the 10 (40%) traits displayed significant additive effects in both the positive and negative directions.

Significant epistatic effects averaged across environments were detected for between two percent and 19% of the double NILs tested (Table 1). The relationship between the frequencies of significant additive and epistatic effects for the same trait was quite variable; ranging from less then half, to more than twice. Similar to the additive effects, epistatic effects in both the positive and negative directions were reported for four of the 10 traits tested. Five and seven percent of the dNILs demonstrated epistasis for days to anthesis and days to silk, respectively. Ear and plant height exhibited epistasis in 17 and 19 percent of the dNILs, respectively.

Effects of specific chromosomal segments: Only the introgression located on 4L did not demonstrate significant additive effects for at least one trait (Table 2). The introgression located on 10L demonstrated significant additive effects for days to anthesis while the regions on 5L and 8S demonstrated significant additive effects for days to silk. The introgression on 8S had an additive effect on days to silk, ear and plant height, erect plants, grain yield, and kernel weight. The introgressions located on 5L, 7L, and 10L were involved in significant additive effects on only one trait each, whereas the introgression on 8S and 10S signficantly affected six and five traits, respectively

All 16 of the introgressions tested were involved in at least one epistatic interaction. Across introgressions, the percentage of traits demonstrating epistatic effects ranged from 40-90%. The introgressions located on 1L, 2L, 8S, and 9S were all involved in epistatic effects for 70% of the tested traits. The introgression involved in the fewest number of epistatic interactions (40%) was located on 5S and was involved in epistasis for ear and plant height only. This introgression displayed signficant additive effects for grain moisture and kernl weight only.

Types of epistasis: All three types of epistasis, synergistic, antagonistic, and non-canceling, were detected among the double-introgression NILs (Table 3). Antagonistic epistasis was by far the most common form of interaction, composing from 40% to 100% of all cases of significant epistasis across traits. Non-canceling epistasis was the second most common form, making up from 0% to 60% of the interactions across traits. However, non-canceling epistasis can be converted to either synergistic or antagonistic epistasis by reassigning genotypic values, and is case-dependent. Synergistic epistasis

occurred for only two traits, ear height and plant height, and only composed 10% and 14% of the interactions, respectively.

The presence of significant additive effects of a chromosomal segment did not appear to be indicative of the likelihood of epistasis in the pairwise combinations including that same segment. Among traits, the range of epistatically interacting pairs involving one segment with significant additive effects was between 10% and 100%. Epistatically interacting pairs in which neither segment had a significant additive effect composed between zero percent and 100% of the interactions, among traits. The percentage of significant interactions between two segments that both had significant additive effects was lowest, ranging from zero percent to 25% among traits.

DISCUSSION

Technical limitations of this study: Our testcross experiment incurs the same problems experienced in the inbred experiment involving these dNIL lines (Tarter 2005). To briefly review, the first problem is the possibility of the loss of a portion of the introgressed segment during the double-introgression line development. Such a loss would bias the additive and epistatic effects estimates. A second problem is non-target introgressions remaining in the single NILs that might have been carried over into the dNILs.

Another consideration in the estimation of additive and epistatic effects in this study is that the single and double-introgression NILs were tested in testcross combinations with Mo17. Only QTL where there are differences between the B73 and Tx303 alleles and when Mo17 is not dominant over the B73 or Tx303 alleles will be detected. However, these gene actions can be considered additive and additive×additive effects because we restrict our reference to the testcross populations (Melchinger 1987).

Finally, the low rates of detection for both significant additive and epistatic effects for the same traits are cause for concern. Because we used $\alpha = 0.05$, we expect five percent of significant tests to be false positives (Type I errors). Fewer significant additive effects were detected than expected by chance for flowering time traits and protein content, suggesting that there may have been no detectable additive effect QTL for these traits.

Trait correlations: Chromosomal regions with significant additive effects on multiple correlated traits were not common. Despite the high correlation between tassel and silk

dates, no introgressions had effects on both traits simultaneously (Table 2). Similarly, only one region had a significant additive effect on both protein and oil content, despite their significant correlation (r = -0.43, Tables 2 and 4). However, only one introgression affecting protein content was detected, whereas three were detected for oil content (Table 2). In contrast, all five QTL regions detected for ear height affected plant height and all were of the same direction. However, an additional four regions (44%), not related to ear height, exhibited a significant additive effect for plant height. While this suggests that some genomic regions might be in common between the two height characters, there must be additional regions involved in the determination of plant height. One of the three (33%) introgressions involved in additive effects for kernel weight were also involved in additive effects for grain yield about what might be expected based on their significant, but low, correlation (r = 0.19, Tables 2 and 4).

In contrast to the additively-acting QTL regions, pairs of introgressions acting epistatically and pleitropically on correlated traits were uncommon. Pairs of chromosomal segments acting epistatically on ear and plant height were the exception. The traits were highly correlated traits (r = 0.71) and had 14 pairs of epistatic introgressions in common. Only two pairs of introgressions that demonstrated epistatic effects were in common for protein and oil content (r = -0.43). Flowering time traits only had two pairs of epistatic combinations in common as well. Despite the significant correlations between remaining traits (plant height and grain yield (r = 0.21); grain yield and kernel weight (r = 0.19)) there were no epistatically-acting introgressed combinations in common among them. Although many pairs of correlated traits were affected by

common chromosome regions involved in epistatic effects (Table 2), the specific pairs of epistatically-acting introgressions were usually not associated with multiple traits.

Relationships between additivity and epistasis: Genetic effects of chromosomal regions were not always limited to either additivity or epistatic effects. The two introgressions involved in the largest number of significant additive effects (8S and 10S) were also involved in the 70% and 80% of the epistatic interactions, respectively. However, one chromosomal introgression, 9S, demonstrated significant epistatic effects for 90% of the traits, but, it was significant for additivity for only 10% of the traits.

Larger percentages of epistatic interactions were noted among the simpler traits compared to the more complex traits. We assume that the number of genes involved in ear/plant height or flowering time are considerably less than those involved in grain yield or kernel characteristics. This is supported by the larger average F-tests for entries for ear and plant height and flowering time traits than for yield or kernel characters (Table 1). Thus, epistatic interactions among fewer loci are easier to detect compared to interactions among the many loci potentially affecting more complex traits like grain yield.

Influence of genotype-by-environment interactions: Genotype-by-environment interactions can confound estimates of additive and epistatic effects derived from evaluations in limited numbers of environments. For all traits except ear and plant height, the percentages of introgressions exhibiting significant additive effects were higher when tests were conducted within individual environments than when averaged across environments (Table 1). Across traits, there was between a 0% and 500% increase

in significant additive effects detected within environments than across environments. A similar pattern was observed for epistatic effects, with the proportion of epistatic effects detected as significant increasing between 0% and 400% (Table 1). This indicates that the genotype-by-environment interaction can affect detection rates and estimates of additive and epistatic effects. The standard analysis, of averaging across all environments, reduces the detection of significant additive and epistatic effects that are correlated with specific environmental conditions, and thus also reduces the overall detection rate.

Associations with previously reported QTL: Despite the different populations being tested, there was some congruency between chromosomal regions displaying signficant additive effects in our study and those reported in the literature. The highly epistatic introgressions associated with days to anthesis and silk, *umc107* (1.10), is close to several other genes reported to be associated with flowering time. The candidate genes ***dwarf8*** (Thornsberry *et al.* 2001) and id1 are both involved in days to flowering under various day-length conditions and are near *umc107*. The high levels of epistasis reported for this region of chromosome one suggest that these various candidate genes are indeed interacting to affect flowering time. Moutiq *et al.* (2002) identified a number of QTL in similar regions to thoses we detected that displayed epistatic effects under long-day conditions in similar regions to those we detected.

Through the compilation of hundreds of data points from multiple maize mapping populations, Khavkin and Coe (1997) attempted to group together previously identified genomic regions of interest. Khavkin and Coe (1997) reported that bins 9.01 and 10.06

contained genes pleiotropically affecting several qualitative traits, and those are regions which contain *umc113* and *umc44a*, two of the most highly epistatic introgressions in our study.

Comparison between inbred and hybrid studies: Somewhat higher proportions of significant additive and epistatic effects were detected among the inbred dNILs compared to dNIL testcrosses, as reported by Tarter (2005) in a companion study involving these dNIL lines. The detection levels of both significant additive and epistatic effects were almost double in the inbred dNILs compared to the testcross dNILs. The means of absolute values and ranges of both additive and epistatic effects were larger in the inbred study as well. One possible explanation could be that in the testcrosses, significant genetic effects could only be detected for loci where the Mo17 allele was not dominant over the B73 and Tx303 alleles.

The number of detected independent significant genetic effects (chromosomal segment-trait interactions) were similar in the inbred (Tarter 2005) and testcross studies. Forty-five examples of significant additive effects were detected in the inbred study (data not shown). A 25% reduction in examples of significantly detected additive effects was found in the testcross study. However, 96 and 94 examples of significant epistatic effects were detected in the inbred and hybrid studies, respectively. A higher level of overall detection of significant additive genetic effects, in inbred as compared to testcross trials, while maintaining a consistent rate of chromosomal segment-trait interactions indicates that the significant additive and epistatic effects were more stable across sets, and thus more prevalent, in inbred field trials than in sets of testcross trials.

The chromosomal regions involved in the largest percentages of significant additive and epistatic effects varied between the inbred and testcross studies. The chromosomal segments affecting the fewest traits in the inbred study were 1L and 4S (Tarter 2005). However, none of those regions were in common with the chromosomal segments affecting the fewst number of traits in the hybrid study. In the inbred study, the introgression on 7S was involved in epistasis for ear and plant height only. However, in the testcross study, it was involved in significant epistatic interactions for 89% of the traits tested.

Significant genotypic × environmental interactions were noticed for all traits tested in both the inbred and hybrid trials. For all traits in both the inbred and hybrid trials, except ear and plant height, more significant genetic effects were detected in the within environment analysis as opposed to the across environment analysis. The range of increase in detection rates of significant additive effects between the two analyses was 0%-600% for the inbred trial and 0% -500% for the hybrid trials. A similar trend was noticed in the detection rates of significant epistatic effects in the inbred and hybrid trials. The range of increase of significant epistatic effects was 0% - 500% for the inbred trials and 0% - 400% for the hybrid trials.

All three types of epistasis were noted in both the inbred and hybrid trials. For both experiments, the rate of synergistic epistasis was below 20%. However, somewhat more antagonstic epistasis was detected in the hybrid trials as opposed to the inbred trials. This could be related to the testcross nature of the hybrid trials and any dominance interaction that might exist between the Tx303 and Mo17 alleles at each introgression site. In spite of the differences in types of epistasis reported, the two studies exhibited

similar findings in terms of proportion of significant epistatic effects involving chromosomal segments with significant additive effects. In both cases, most of the epistatic interactions were detected between chromosomal segments that did not display significant additive effects singly. Such epistatic effects are likely to remain undetected using typical QTL mapping strategies such as multiple interval mapping (Kao *et al.* 1999), which model epistasis only between QTL with significant main effects.

Significant additive and epistatic effects were detected in the single and double-introgression near-isogenic lines in both the inbred and testcross trials. However, differences in detection rates and effects estimates between the two studies warrant testing the dNILs in both in the inbred line and hybrid state. Variability of effect estimates between inbred and hybrid combinations could lead to poor predictions of responses to marker-assisted selection of QTL detected in only one type of reference population.

Conclusions: Through the use of double-introgression near-isogenic lines, we demonstrated statistical evidence of epistatic interactions between introgressed regions within near-isogenic lines. Overall, epistatic interactions are less common and have smaller magnitudes than additive effects. Those epistatic interactions that were detected were most often antagonistic in nature. This suggests that predictions of responses to marker-assisted selection based on additive effects are much more likely to be overestimated than underestimated if epistasis occurs between QTL. Further, most epistatic effects involved at least one segment that had no significant additive effect.

This result also suggests that QTL-by-genetic background interactions are likely to be more common than QTL-by-QTL epistasis.

These results are in agreement with Hallauer and Miranda (1981), Hinze and Lamkey (2003), and Mihaljevic *et al.* (in review) that overall, epistasis is less important than additive genetic effects for quantitative traits in maize, but that there may be specific populations, traits, and chromosomal regions for which epistatic effects are important and can affect the genetic architecture and response to selection. These results are also in agreement with Eshed and Zamir (1996) who appear to have produced the only other populations of double-introgression near-isogenic lines developed to study epistatic interactions. They also reported significant epistatic itneractions for between 20 and 42% of the introgression pairs for five fruiting traits of tomato. They reported that the epistasis was usually less-than-additive in nature and most often included introgression segments that did not display significant additive effects singly. These results are in agreement with those reported here and confirm the hypothesis that epistatic effects are indeed present in populations and usually go undetected because of the statistical procedures and analysis methods used to estimate them.

These findings tend to support the theory of epistasis as proposed by Wright's shifting balance theory (Wright 1931). The tested population was comprised of a small, local population and the estimates of epistasis were conducted on allelic combinations, even though they were artifically developed (Brodie 2000). The ability of epistatic interactions to move a subpopulation through a fitness valley and cause a temporary response to selection was exemplified by the superior phenotypic performance of some of the double-introgression lines compared to B73 while also exhibiting significant epistatic

interactions (Holland 2001; Wright 1968). This implies that the genetic effects of positive epistatic interactions could be captured and developed into specific cultivars for release.

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TABLE 1. Range, mean of absolute values, and proportion significant of additive and epistatic effects identified in single-introgression and double-introgression near-isogenic lines evaluated in testcross experiments in 8 North Carolina environments.

	Days to anthesis[a]	Days to silk[a]	Ear height	Plant height	Erect plants	Grain yield	Grain moisture	Kernel weight	Protein content[b]	Oil content[b]
Additive Effects										
	d	d	m	m	%	T ha^{-1}	%	g	%	%
Range	-1.5	2.8	-0.09,0.08	-0.09,0.10	-13,-9	-0.9,-0.6	-0.75,0.81	-0.03,0.03	-1.02	0.4,0.7
Mean of absolute values	1.5	2.8	0.07	0.08	11	0.7	0.75	0.03	1.02	0.6
Introgressed chromosome segments with significant additive effects										
	%	%	%	%	%	%	%	%	%	%
Additive effects averaged across environments [58 tests]										
	2	2	16	28	7	5	5	7	2	7
Additive effects estimated within individual environments [115-436 tests]										
	5	10	13	16	9	8	11	7	9	7

TABLE 1. Continued.

Epistatic Effects										
	d	d	m	m	%	T ha^{-1}	%	g	%	%
Range	-0.8, 1.4	-1.9,1.3	-0.04,0.06	-0.05,0.11	7,8	0.4,0.6	-0.7,0.5	-0.02,0.03	0.5,0.8	-0.5,-0.2
Mean of absolute values	1.1	1.3	0.05	0.05	8	0.5	0.5	0.02	0.7	0.3
Double-introgression NILs demonstrating significant epistatic effects										
	%	%	%	%	%	%	%	%	%	%
Epistatic effects averaged across environments [113 tests]										
	5	7	17	19	2	3	6	4	5	7
Epistatic effects estimated within individual environments [209-794 tests]										
	11	10	10	13	8	6	8	7	10	8
Mean F-values for genotypes averaged in the combined analysis across sets										
Entry	3.74	4.06	5.43	6.23	3.00	1.31	3.61	2.75	1.34	2.24

[a] Days to anthesis and silk are recorded at Clayton only.

[b] Protein and oil data are from Clayton and Plymouth in 2003 only.

TABLE 2. Summary of introgressed regions, their RFLP probes, and percentage of sets containing the sNIL carrying the introgression in which the additive effect was significant for each trait, based on NIL testcrosses to Mo17 evaluated in 8 North Carolina environments.

Genomic Location	RFLP Clone	Days to Anthesis	Days to Silk	Ear Height	Plant Height	Erect Plants	Grain Yield	Grain Moisture	Kernel Weight	Protein Content	Oil Content
1L	umc107				25	50					
2L	umc122				75						
3S	umc32a				67						
4S	bnlg5.46				50						
4L	bnlg8.23										
5S	umc147a							25	25		
5L	umc68		33								33
6C	umc21			67	100						67
6S	umc85			67	33			33			
7S	umc116			50	50						
7L	umc168						25				
8S	umc32b		25	50	25	25	25		50		
9S	umc113					25					
9L	bnlg14.28						33				
10S	umc155				25			25	25	25	25

TABLE 2. Continued.

10L	umc44a	33

TABLE 3. Summary of introgressed regions, their RFLP probes, and percentage of dNILs carrying an introgression in which significant epistatic effects were detected for each trait, based on NIL testcrosses to Mo17 evaluated in 8 North Carolina environments.

Genomic Location	RFLP Clone	Days to Anthesis	Days to Silk	Ear Height	Plant Height	Erect Plants	Grain Yield	Grain Moisture	Kernel Weight	Protein Content	Oil Content
1L	umc107	21	7	21	21				7	7	7
2L	umc122	7	20	13	7		7	7	7		7
3S	umc32a			21	36				7	14	7
4S	bnlg5.46	7	7	14	7		7				
4L	bnlg8.23			15	8			8	8		8
5S	umc147a			7	7			21	7		
5L	umc68			13	20	27				7	7
6C	umc21		7	29	14				7	14	7
6S	umc85		7	40	47			13			
7S	umc116	17		33	27					7	14
7L	umc168		7		20		7	7			7
8S	umc32b	13	20			7	13	13	7		7
9S	umc113	7	7	7	14	7		7	7	7	7
9L	bnlg14.28			21	29	14			7	7	
10S	umc155	14	7	14	14			7	7	7	21

TABLE 3. Continued.

10L	umc44a	8	8	8	15	8	15

TABLE 4. Summary of types of epistasis exhibited by testcrossed double-introgression NILs with significant epistatic effects and significant parental additive effects combined over all sets in 8 North Carolina environments.

	Days to anthesis	Days to silk	Ear height	Plant height	Erect plants	Grain yield	Grain moisture	Kernel weight	Protein content	Oil content
	%	%	%	%	%	%	%	%	%	%
Synergistic epistasis	0	0	10	14	0	0	0	0	0	0
Antagonistic epistasis	100	100	74	86	100	67	86	40	100	100
Non-canceling epistasis	0	0	16	0	0	33	14	60	0	0
Proportion of significant epistatic effects involving 0, 1, or 2 chromosomal segments with significant additive effects										
No. of segments with significant additive effects	%	%	%	%	%	%	%	%	%	%
0	80	50	37	67	0	0	43	40	100	72
1	20	50	58	10	100	100	43	60	0	14
2	0	0	5	24	0	0	14	0	0	14

TABLE 5. Correlation coefficients between agronomic traits estimated from double-introgression NIL trait mean deviations.

	Days to Silk	Ear Height	Plant Height	Erect Plants	Grain Yield	Grain Moisture	Kernel Weight	Protein Content	Oil Content
Days to Anthesis	0.57**	0.21*	0.15*	0.04	-0.05	0.16*	0.04	0.08	-0.16*
Days to Silk		0.06*	0.15**	0.24*	0.15	0.09	-0.03	-0.06	0.07
Ear Height			0.71**	-0.30**	0.07	-0.13	-0.01	0.08	-0.14
Plant Height				-0.08	0.21*	-0.08	0.21*	0.11	-0.17*
Erect Plants					0.13	-0.06	0.11	-0.02	-0.07
Grain Yield						0.02	0.19*	-0.03	0.20*
Grain Moisture							-0.16*	-0.23*	0.08
Kernel Weight								0.18*	-0.19*
Protein Content									-0.43**

* Indicates significant correlation at $P \leq 0.05$.

** Indicates significant correlation at $P \leq 0.001$.

IV. Appendices

Appendix A : Double-introgression near-isogenic line development

Figure A1. Diagram of 16 introgressed regions of Tx303 germplasm into B73 background. Crosshatch marks represent bin sizes approximately 20cm (Davis et al. 1999).

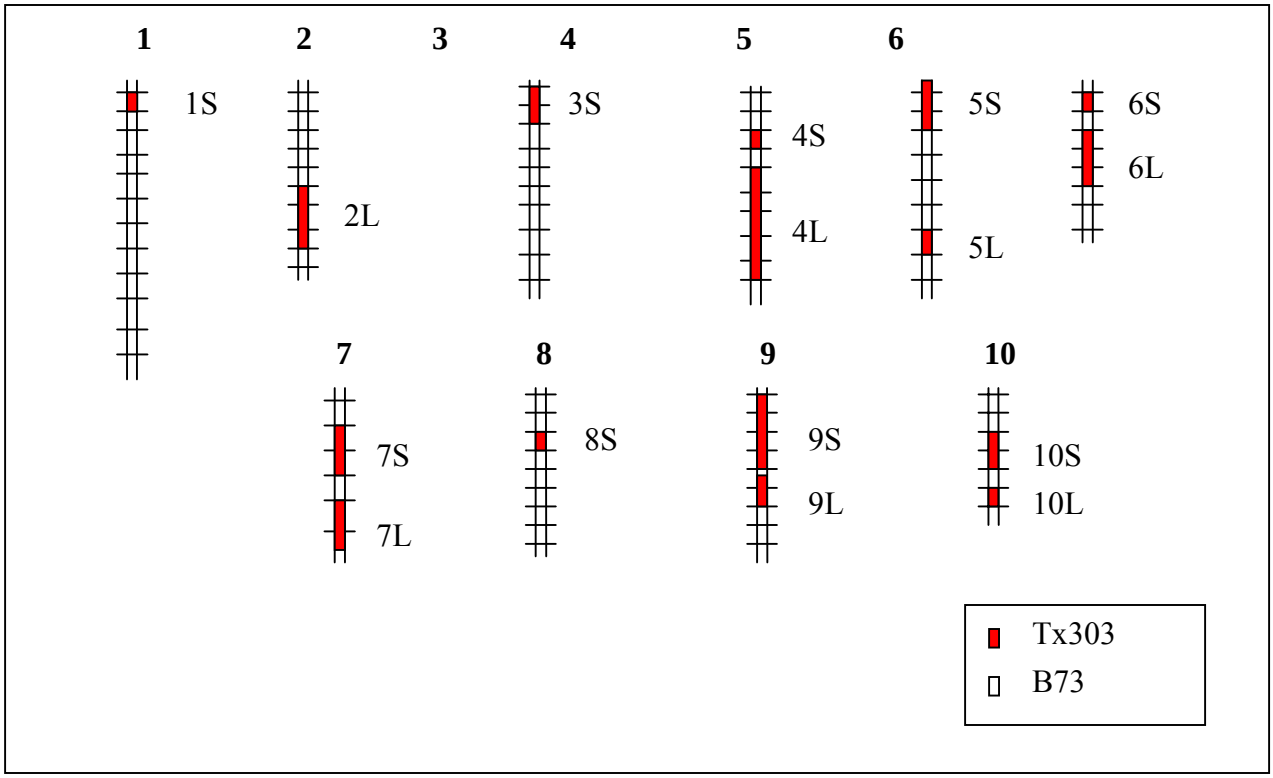

Figure A2. Schematic diagram of breeding scheme including marker-assisted selection for development of double-introgression NILs generated with B73 and Tx303.

Summer 2000, Clayton NC

Single introgression NIL_1 × Single introgression NIL_2

↓

F1 progeny

Winter 2000-2001, Homestead FL

F_1 progeny

⊗

↓

F_2 progeny

Summer 2001, Clayton NC

F_2 individual plants :MAS implemented at two loci

⊗

↓

F_3 progeny : selecting individual ears/row

Winter 2001-2002, Homestead FL

F_3 individual plants : MAS implemented at second locus where needed

F_4 progeny : selecting individual ears/row

Summer 2002, Clayton NC

Identified dNILs (F_4 plants) : replicated inbred trial, selfed seed increase, testcrossed to Mo17

Unidentified dNILs F_4 individual plants : MAS implemented at one locus

F_5 progeny : selecting individual ears/row

Winter 2002-2003, Homestead FL

Identified dNILs (F_5 plants) : selfed seed increase,

testcrossed to Mo17

Unidentified dNILs (F_3 to F_5) individual plants :

MAS implemented at one locus

Selected progeny : selecting individual ears/row

Figure A3. Diallel chart indicating all single-introgression NILs crossed in all pairwise combinations to generate double-introgression NILs. Introgressed segments are along the axes and the numbers in the cells indicate the number of independently derived dNILs.

	10S	**4L**	**1L**	**4S**	**7L**	**7S**	**8S**	**9S**	**2L**	**6C**	**10L**	**6S**	**5L**	**3S**	**9L**
5S	1	3	1	1	1	1	2	1	1	1	0	1	1	1	1
10L		1	1	1	1	1	1	0	1	1	1	1	1	1	1
4L			1	1	1	3	1	1	3	1	0	2	2	0	1
1L				1	1	1	1	1	1	0	1	1	1	1	1
4S					1	1	1	1	1	1	1	1	1	1	0
7L						1	1	1	1	1	1	1	1	1	1
7S							1	2	1	1	2	1	2	1	1
8S								1	1	1	1	1	1	1	1
9S									1	1	1	1	1	1	1
2L										2	1	1	1	1	1
6C											1	1	2	1	1
10L												1	1	1	1
6S													2	1	1
5L														1	1
3S															1

Appendix B : Double-introgression NIL phenotypic performance means

Table A1. Least square means for single and double-introgression near-isogenic lines from set 1 evaluated as inbreds, *per se,* in 6 North Carolina environments.

dNIL Line	Pedigree	Seed Source	Stand	Days to Tassel	Days to Silk	Ear Height	Plant Height	Grain Yield	Grain Moisture	Kernel	Protein	Oil
			%	d	d	m	m	g	%	g	%	%
1	(TBBC3-02x11)	1266/02	0.94	68.21	71.19	0.82	1.82	1184.95	6.80	0.23	12.82	2.79
2	(TBBC3-02x13-1)	1304/02	0.94	67.53	67.79	0.75	1.67	1464.52	5.15	0.22	15.34	3.24
3	(TBBC3-02x13-2)	1315/02	0.91	66.28	68.29	0.75	1.76	1333.32	5.80	0.22	14.03	3.30
4	(TBBC3-02x13-3)	1314/02	0.76	67.21	67.94	0.78	1.72	1260.30	3.82	0.20	13.16	3.45
5	(TBBC3-02x15)	1255/02	0.94	67.53	70.29	0.73	1.73	1350.64	5.30	0.22	14.33	2.93
6	(TBBC3-02x16)	5518/02	0.98	68.78	70.04	0.83	1.87	868.99	5.47	0.24	14.96	2.45
7	(TBBC3-02x19)	1285/02	0.65	68.03	70.79	0.80	1.85	1324.55	6.30	0.23	13.28	2.63
19	(TBBC3-11x13)	1309/02	0.98	67.46	68.69	0.73	1.73	1083.74	6.78	0.19	13.77	3.21
20	(TBBC3-11x15)	1347/02	0.93	66.78	68.54	0.68	1.56	1193.95	5.66	0.21	12.97	2.92
21	(TBBC3-11x16)	2962/02	1.00	67.85	69.89	0.78	1.77	1260.69	5.80	0.24	13.94	2.89
22	(TBBC3-11x19)	1354/02	0.93	66.60	68.39	0.74	1.75	1151.47	7.95	0.21	13.47	2.89
32	(TBBC3-13x15)	2988/02	1.00	67.03	69.79	0.67	1.59	1198.77	3.98	0.16	14.34	3.64
33	(TBBC3-13x16)	2993/02	0.84	66.21	67.69	0.81	1.70	1262.65	6.78	0.21	12.04	3.07
34	(TBBC3-13x19)	1292/02	0.94	66.71	67.44	0.74	1.69	1561.80	5.80	0.23	13.17	2.98

TABLE A1. Continued.

51	(TBBC3-15x16)	5529/02	1.00	67.78	70.04	0.93	1.87	1122.00	7.46	0.18	13.88	2.69
52	(TBBC3-15x19)	5530/02	0.96	67.21	69.19	0.74	1.74	1270.32	6.45	0.21	12.97	2.77
63	(TBBC3-16x19)	3058/02	0.99	66.60	69.14	0.81	1.83	1412.42	6.64	0.23	13.12	2.83
137	TBBC3-2	198/02	0.95	67.64	69.59	0.78	1.80	1052.22	6.13	0.23	13.86	2.97
138	TBBC3-11	199/02	0.97	68.39	71.09	0.70	1.65	971.37	9.62	0.21	11.82	3.14
139	TBBC3-13	200/02	0.98	66.28	67.29	0.71	1.60	1187.94	6.13	0.20	15.30	2.70
140	TBBC3-15	201/02	0.92	67.46	69.44	0.75	1.68	1230.57	6.47	0.21	14.13	3.02
141	TBBC3-16	124/02	0.96	67.53	69.04	0.78	1.78	1232.00	6.96	0.22	12.71	3.15
142	TBBC3-19	203/02	0.95	67.53	68.79	0.81	1.83	1355.19	6.63	0.23	12.89	2.82
153	B73	1/03	0.94	67.23	68.64	0.74	1.70	1255.84	5.76	0.22	13.37	3.06
157	Tx303	45/03	0.99	74.96	77.19	0.96	1.82	801.75	14.26	0.30	13.07	2.94
162	TBBC3-27	135/02	0.98	66.71	68.19	0.72	1.71	1423.00	5.96	0.21	12.78	3.05
	Mean		0.94	67.60	69.40	0.77	1.74	1223.65	6.53	0.22	13.52	2.98
	LSD (0.05)		0.13	1.97	2.31	0.06	0.08	367.87	3.31	0.04	2.14	0.41

TABLE A2. Least square means for single and double-introgression near-isogenic lines from set 2 evaluated as inbreds, *per se,* in 6 North Carolina environments.

dNIL	Line	Pedigree	Seed Source	Stand	Days to Tassel	Days to Silk	Ear Height	Plant Height	Grain Yield	Grain Moisture	Kernel	Protein	Oil
				%	d	d	m	m	g	%	g	%	%
8		(TBBC3-02x24)	5519/02	0.91	67.06	70.05	0.86	1.85	1462.95	8.25	0.23	12.29	2.88
9		(TBBC3-02x42-1)	1284/02	0.59	66.87	68.58	0.74	1.79	1092.95	6.77	0.21	12.72	2.63
10		(TBBC3-02x42-2)	1302/02	0.90	66.60	68.60	0.79	1.83	1513.97	7.75	0.22	12.66	2.83
11		(TBBC3-02x47)	1313/02	0.72	67.65	68.96	0.76	1.80	948.91	7.75	0.23	13.33	2.83
12		(TBBC3-02x49)	1311/02	0.79	68.47	69.19	0.73	1.79	1237.25	10.75	0.23	14.20	3.22
23		(TBBC3-11x24)	1259/02	0.92	66.35	68.89	0.71	1.64	1271.54	16.50	0.23	13.38	3.08
24		(TBBC3-11x42)	1342/02	0.96	66.54	69.69	0.73	1.77	1108.03	9.75	0.22	12.97	2.90
25		(TBBC3-11x49)	5523/02	0.97	68.32	70.15	0.77	1.75	896.92	10.25	0.21	13.88	3.05
35		(TBBC3-13x24-1)	1280/02	0.83	65.57	68.70	0.75	1.63	1278.70	10.25	0.19	13.08	2.88
36		(TBBC3-13x24-2)	1337/02	0.91	66.81	68.35	0.74	1.65	1130.08	8.93	0.21	12.09	2.79
37		(TBBC3-13x24-3)	1343/02	0.87	66.72	68.20	0.74	1.61	1366.14	9.00	0.21	11.15	2.78
38		(TBBC3-13x42)	1272/02	0.92	67.73	68.97	0.81	1.82	1464.03	10.75	0.23	12.72	3.03
39		(TBBC3-13x47)	1291/02	0.89	65.55	65.49	0.78	1.70	1144.49	6.23	0.22	12.19	3.16
40		(TBBC3-13x49-1)	1247/02	0.87	65.49	67.40	0.66	1.50	1066.50	7.50	0.22	15.03	3.50

TABLE A2. Continued.

41	(TBBC3-13x49-2)	1271/02	0.86	65.98	68.58	0.74	1.75	1081.47	9.50	0.23	15.19	2.81
42	(TBBC3-13x49)	1294/02	0.95	67.75	68.70	0.77	1.76	1154.47	8.50	0.22	13.56	3.42
53	(TBBC3-15x24)	1276/02	0.93	66.50	69.86	0.76	1.72	1069.82	13.25	0.21	12.17	2.95
54	(TBBC3-15x42)	5531/02	0.96	65.54	68.35	0.86	1.75	1506.33	11.75	0.20	13.14	3.22
55	(TBBC3-15x47)	5522/02	0.81	64.44	67.11	0.69	1.60	1010.32	7.40	0.22	13.39	2.49
56	(TBBC3-15x49)	1256/02	0.92	67.50	69.23	0.78	1.78	917.07	11.00	0.23	13.89	2.61
137	TBBC3-2	198/02	0.75	68.34	70.32	0.80	1.78	1130.23	8.50	0.24	14.14	2.89
138	TBBC3-11	199/02	0.97	67.58	70.55	0.75	1.70	1195.09	14.50	0.20	12.78	2.99
139	TBBC3-13	200/02	0.92	65.85	67.59	0.69	1.62	1131.86	7.75	0.21	13.13	3.20
140	TBBC3-15	201/02	0.86	65.77	69.05	0.77	1.70	1402.98	10.00	0.21	13.16	3.05
143	TBBC3-24	204/02	0.96	65.97	68.75	0.67	1.61	1437.93	10.75	0.21	11.54	3.27
144	TBBC3-42	150/02	0.83	66.35	69.20	0.86	1.91	1466.89	11.50	0.19	12.16	3.29
145	TBBC3-47	207/02	0.89	65.71	66.64	0.76	1.74	1134.82	7.23	0.23	12.91	2.98
146	TBBC3-49	157/02	0.86	67.79	68.79	0.81	1.84	959.87	10.00	0.22	14.20	2.83
153	B73	1/03	0.89	66.75	68.65	0.76	1.76	1378.73	8.42	0.22	13.13	3.07
157	Tx303	45/03	0.91	74.19	74.88	0.92	1.78	713.97	21.75	0.31	13.71	2.81
	Mean		0.88	66.93	68.92	0.77	1.73	1189.14	10.07	0.22	13.13	2.98
	LSD (0.05)		0.15	1.88	2.26	0.05	0.07	343.31	2.93	0.02	1.35	0.45

TABLE A3. Least square means for single and double-introgression near-isogenic lines from set 3 evaluated as inbreds, *per se,* in 6 North Carolina environments.

dNIL Line	Pedigree	Seed Source	Stand	Days to Tassel	Days to Silk	Ear Height	Plant Height	Grain Yield	Grain Moisture	Kernel	Protein	Oil
			%	d	d	m	m	g	%	g	%	%
64	(TBBC3-16x24)	3071/02	0.67	69.88	71.67	0.72	1.64	777.07	10.34	0.22	14.63	2.64
65	(TBBC3-16x42)	3103/02	0.77	69.09	71.48	0.88	1.91	875.90	11.91	0.26	15.13	2.50
66	(TBBC3-16x47)	3113/02	0.67	69.79	70.34	0.81	1.81	987.57	9.49	0.21	14.68	2.63
67	(TBBC3-16x49)	3116/02	0.71	67.86	69.13	0.73	1.77	937.36	10.59	0.23	14.45	2.58
76	(TBBC3-19x24)	1333/02	0.79	67.07	69.26	0.77	1.66	1297.46	10.84	0.21	12.16	2.91
77	(TBBC3-19x42)	5785/02	0.93	66.43	68.65	0.90	1.90	1569.10	10.16	0.22	12.78	2.90
78	(TBBC3-19x47)	1250/02	0.59	66.63	67.42	0.79	1.79	1166.66	10.59	0.21	12.93	2.65
79	(TBBC3-19x49)	1310/02	0.72	67.58	69.31	0.79	1.82	1087.57	13.09	0.23	14.00	2.93
86	(TBBC3-24x42)	1334/02	0.74	66.51	69.65	0.76	1.68	1399.37	7.84	0.23	12.33	3.15
87	TBBC3-24x47-1	215/03	0.78	67.86	69.71	0.68	1.54	1153.72	12.59	0.20	.	.
88	TBBC3-24x47-2	216/03	0.66	65.72	69.28	0.77	1.75	1747.37	14.52	0.23	.	.
89	(TBBC3-24x49)	1338/02	0.73	68.79	69.82	0.72	1.65	1193.56	11.84	0.23	13.61	2.92
98	(TBBC3-42x47)	1340/02	0.76	66.69	67.99	0.92	1.89	1417.21	11.34	0.23	13.55	2.95
99	(TBBC3-42x49)	3415/02	0.67	67.81	68.99	0.78	1.88	1043.65	13.59	0.22	14.73	3.48
106	(TBBC3-47x49)	5850/02	0.85	65.20	67.52	0.74	1.76	1019.06	8.84	0.23	14.68	2.85
141	TBBC3-16	202/02	0.75	68.12	68.11	0.77	1.78	1398.04	9.45	0.22	12.69	2.91

TABLE A3. Continued.

142	TBBC3-19	203/02	0.81	67.12	69.41	0.80	1.79	1148.37	10.34	0.22	13.41	2.74
143	TBBC3-24	204/02	0.83	66.27	69.60	0.71	1.57	1419.52	11.59	0.21	12.25	3.14
144	TBBC3-42	206/02	0.79	67.08	69.75	0.88	1.92	1557.26	12.84	0.21	13.33	3.01
145	TBBC3-47	207/02	0.76	67.49	67.89	0.74	1.68	949.66	8.09	0.23	13.44	2.58
146	TBBC3-49	208/02	0.79	67.88	69.22	0.81	1.85	798.21	11.09	0.22	14.82	2.66
153	B73	1/03	0.73	67.37	69.29	0.75	1.71	1320.99	9.34	0.22	13.66	2.90
157	Tx303	45/03	0.83	75.02	77.02	1.00	1.85	685.86	24.34	0.31	12.52	3.06
163	TBBC3-54	162/02	0.68	66.19	67.99	0.87	1.88	955.87	7.84	0.23	15.53	2.97
	Mean		0.75	67.73	69.52	0.80	1.77	1162.77	11.36	0.23	13.70	2.86
	LSD (0.05)		0.22	2.50	2.35	0.07	0.09	420.71	3.84	0.02	1.47	0.56

TABLE A4. Least square means for single and double-introgression near-isogenic lines from set 4 evaluated as inbreds, *per se,* in 6 North Carolina environments.

dNIL Line	Pedigree	Seed Source	Stand	Days to Tassel	Days to Silk	Ear Height	Plant Height	Grain Yield	Grain Moisture	Kernel	Protein	Oil
			%	d	d	m	m	g	%	g	%	%
13	(TBBC3-02x51)	1344/02	0.88	70.28	73.25	0.81	1.80	692.93	14.88	0.25	14.03	3.24
15	(TBBC3-02x63)	1254/02	0.77	67.62	69.52	0.76	1.72	1185.62	13.27	0.25	14.41	2.87
26	(TBBC3-11x51)	2971/02	0.93	66.23	69.75	0.71	1.78	1072.47	15.34	0.20	14.67	3.03
27	(TBBC3-11x62)	1299/02	0.84	67.69	69.99	0.77	1.75	1177.71	11.19	0.21	13.84	2.86
28	(TBBC3-11x63)	2975/02	0.95	68.91	71.74	0.74	1.60	1067.80	10.68	0.20	14.19	3.19
43	(TBBC3-13x51)	1275/02	0.81	67.15	68.51	0.74	1.62	1181.37	10.48	0.22	13.68	3.54
45	TBBC3-13x63	173/03	0.84	65.94	69.33	0.77	1.68	1308.01	13.86	0.23	.	.
58	(TBBC3-15x62)	3048/02	0.93	68.17	75.02	0.74	1.78	280.92	16.92	0.20	17.20	3.09
59	(TBBC3-15x63)	1279/02	0.59	67.26	69.75	0.69	1.53	1262.87	8.43	0.24	13.70	2.90
68	(TBBC3-16x51)	3126/02	0.91	65.99	68.50	0.71	1.70	1147.05	12.46	0.22	13.55	3.34
69	(TBBC3-16x62)	3131/02	0.67	67.03	69.00	0.79	1.74	1281.42	8.03	0.24	13.43	3.03
70	(TBBC3-16x63)	3148/02	0.75	67.88	69.48	0.79	1.76	1209.68	11.13	0.24	14.46	3.00
137	TBBC3-2	198/02	0.81	67.48	69.75	0.77	1.77	1461.03	9.68	0.24	13.15	3.16
138	TBBC3-11	199/02	0.91	67.55	69.98	0.72	1.70	979.62	15.10	0.20	14.27	3.04
139	TBBC3-13	200/02	0.85	67.01	68.25	0.71	1.61	1291.54	10.52	0.23	13.36	3.26
140	TBBC3-15	201/02	0.77	66.74	69.50	0.75	1.70	1361.92	12.82	0.21	12.82	2.92

TABLE A4. Continued.

141	TBBC3-16	202/02	0.90	68.15	68.52	0.75	1.77	1088.08	8.85	0.23	13.46	2.98
147	TBBC3-51	209/02	0.68	66.28	68.75	0.70	1.62	1148.69	9.48	0.21	14.18	3.72
148	TBBC3-62	210/02	0.82	67.51	69.53	0.75	1.74	1167.21	9.70	0.22	13.43	2.92
149	TBBC3-63	211/02	0.95	67.03	68.97	0.66	1.56	1369.82	10.14	0.23	13.38	3.31
153	B73	1/03	0.77	66.67	68.56	0.75	1.73	1373.14	10.26	0.23	12.98	2.94
157	Tx303	45/03	0.87	73.04	75.47	0.91	1.82	857.14	22.52	0.31	12.55	3.03
	Mean		0.83	67.62	70.05	0.75	1.70	1134.82	12.08	0.23	13.85	3.11
	LSD (0.05)		0.17	2.12	2.86	0.06	0.08	304.34	4.73	0.02	1.26	0.38

TABLE A5. Least square means for single and double-introgression near-isogenic lines from set 5 evaluated as inbreds, *per se,* in 6 North Carolina environments.

dNIL	Line Pedigree	Seed Source	Stand	Days to Tassel	Days to Silk	Ear Height	Plant Height	Grain Yield	Grain Moisture	Kernel	Protein	Oil
			%	d	d	m	m	g	%	g	%	%
80	(TBBC3-19x51)	3640/02	0.99	68.01	69.84	0.69	1.67	733.62	15.67	0.21	14.47	3.30
81	(TBBC3-19x62)	3230/02	0.98	68.31	69.50	0.78	1.78	1038.89	11.61	0.20	12.65	2.83
82	(TBBC3-19x63)	1336/02	0.97	66.50	68.28	0.74	1.72	1490.53	10.65	0.23	13.60	2.93
90	(TBBC3-24x51)	1283/02	0.82	66.30	67.95	0.74	1.68	1306.63	13.71	0.20	13.38	3.22
91	(TBBC3-24x62)	1248/02	0.78	68.10	70.16	0.75	1.72	1199.76	13.39	0.21	13.17	2.98
92	(TBBC3-24x62)	1301/02	0.93	68.64	70.93	0.81	1.69	1228.92	10.93	0.22	13.19	2.93
93	(TBBC3-24x63)	1325/02	1.00	66.58	69.07	0.74	1.54	1369.30	15.42	0.20	12.24	2.85
100	(TBBC3-42x51)	1295/02	0.90	68.24	70.39	0.75	1.71	724.64	7.86	0.18	14.11	3.70
101	(TBBC3-42x62)	1262/02	0.95	68.94	72.20	0.82	1.88	1175.62	11.14	0.21	13.75	2.60
102	(TBBC3-42x63)	1289/02	0.89	68.89	72.15	0.74	1.65	709.15	12.33	0.18	14.48	2.73
107	(TBBC3-47x51)	1357/02	0.71	67.02	67.66	0.71	1.61	850.86	5.96	0.19	13.59	2.92
108	(TBBC3-47x62)	3441/02	0.91	67.18	68.00	0.76	1.78	1208.29	12.55	0.22	14.94	2.22
109	(TBBC3-47x63)	5862/02	1.00	66.59	68.41	0.71	1.67	1450.24	12.36	0.22	13.86	3.01
113	(TBBC3-49x51-1)	1270/02	0.78	69.05	71.32	0.74	1.72	780.97	11.74	0.20	17.26	3.45
114	(TBBC3-49x51-2)	1316/02	0.86	68.71	71.35	0.76	1.77	871.45	15.11	0.22	15.43	3.87
115	(TBBC3-49x62)	3454/02	0.89	68.18	69.24	0.90	2.09	870.17	12.15	0.23	14.74	2.63

TABLE A5. Continued.

116	(TBBC3-49x63)	5875/02	1.00	64.39	65.20	0.62	1.52	1120.58	7.27	0.22	12.60	2.97
142	TBBC3-19	203/02	0.99	67.50	69.21	0.75	1.74	1325.15	12.96	0.22	13.68	2.60
143	TBBC3-24	204/02	0.99	68.04	69.93	0.75	1.61	1498.60	11.96	0.21	12.64	3.20
144	TBBC3-42	206/02	0.98	68.27	70.01	0.92	1.96	1657.09	13.05	0.20	12.86	3.27
145	TBBC3-47	207/02	1.00	67.78	68.53	0.71	1.69	1048.13	10.80	0.24	14.54	2.40
146	TBBC3-49	208/02	0.93	68.48	69.54	0.78	1.79	891.14	11.83	0.20	14.45	2.73
147	TBBC3-51	209/02	0.99	66.37	69.14	0.64	1.57	954.86	8.58	0.19	13.75	3.74
148	TBBC3-62	210/02	0.98	67.55	69.35	0.79	1.82	1301.68	10.27	0.16	12.74	2.85
149	TBBC3-63	211/02	0.95	66.46	68.50	0.69	1.54	1408.40	10.05	0.22	11.48	3.04
153	B73	1/03	1.00	67.33	69.11	0.75	1.73	1361.51	10.17	0.22	13.40	2.94
157	Tx303	45/03	0.99	75.08	77.19	0.97	1.87	858.24	25.90	0.30	12.42	2.79
	Mean		0.93	67.87	69.71	0.76	1.72	1127.20	12.05	0.21	13.68	2.99
	LSD (0.05)		0.08	1.69	2.55	0.06	0.09	350.39	5.12	0.05	1.29	0.54

TABLE A6. Least square means for single and double-introgression near-isogenic lines from set 6 evaluated as inbreds, *per se,* in 6 North Carolina environments.

dNIL	Line Pedigree	Seed Source	Stand	Days to Tassel	Days to Silk	Ear Height	Plant Height	Grain Yield	Grain Moisture	Kernel	Protein	Oil
			%	d	d	m	m	g	%	g	%	%
120	(TBBC3-51x62)	3486/02	0.86	67.69	70.17	0.76	1.65	1056.43	9.44	0.21	14.53	3.72
121	(TBBC3-51x63)	3494/02	0.84	72.18	73.41	0.64	1.53	512.51	17.36	0.23	14.75	3.13
122	(TBBC3-51x71-1)	1322/02	0.66	67.08	68.80	0.67	1.62	832.79	10.84	0.22	16.06	3.50
123	(TBBC3-51x71-2)	1245/02	0.89	67.88	68.99	0.69	1.70	997.85	10.81	0.23	14.31	3.35
124	(TBBC3-51x81)	3524/02	0.97	67.69	69.89	0.72	1.65	1094.73	10.85	0.23	14.29	3.59
125	(TBBC3-51x87)	3532/02	1.00	66.99	68.62	0.75	1.72	1187.22	9.23	0.21	13.74	3.54
126	(TBBC3-62x63)	5550/02	0.98	68.82	71.27	0.76	1.64	1209.09	8.85	0.22	11.99	3.05
127	(TBBC3-62x71)	1260/02	0.93	70.02	71.54	0.79	1.85	1144.84	11.44	0.23	13.48	3.13
128	(TBBC3-62x81)	3558/02	0.76	70.11	71.97	0.82	1.80	982.74	12.42	0.23	14.15	2.84
129	(TBBC3-62x87)	5885/02	1.00	68.72	69.56	0.85	1.83	1287.08	10.57	0.22	14.08	2.90
130	(TBBC3-63x71-1)	1303/02	0.90	69.19	71.05	0.74	1.72	1133.22	13.39	0.24	14.15	3.09
131	(TBBC3-63x71-2)	1305/02	0.94	67.50	70.75	0.71	1.65	1071.84	10.40	0.23	13.47	3.26
132	(TBBC3-63x81)	1326/02	0.85	69.99	71.20	0.70	1.61	832.99	8.80	0.25	13.43	2.93
133	(TBBC3-63x87)	5551/02	0.99	68.74	70.15	0.84	1.81	1449.79	11.45	0.23	13.68	3.02
134	(TBBC3-71x81)	3608/02	0.73	69.54	71.34	0.75	1.71	603.97	11.84	0.22	14.56	2.64
135	(TBBC3-71x87)	3617/02	1.00	66.52	68.72	0.76	1.82	1142.03	14.34	0.26	15.22	3.50

TABLE A6. Continued.

136	(TBBC3-81x87)	1327/02	0.84	68.94	69.82	0.76	1.72	900.17	8.61	0.24	14.21	2.81
147	TBBC3-51	209/02	0.93	66.72	70.40	0.68	1.66	1046.01	9.90	0.20	13.47	3.58
148	TBBC3-62	210/02	0.98	69.19	71.18	0.77	1.74	954.95	11.86	0.22	13.34	2.84
149	TBBC3-63	211/02	0.99	68.18	69.87	0.67	1.54	1340.58	7.56	0.23	12.50	3.13
150	TBBC3-71	213/02	0.96	66.98	69.23	0.74	1.71	1033.68	12.27	0.24	14.34	3.19
151	TBBC3-81	214/02	0.87	67.97	71.44	0.74	1.77	831.00	16.04	0.24	14.89	2.93
152	TBBC3-87	215/02	0.96	68.33	68.31	0.80	1.79	1508.98	11.14	0.22	12.44	3.09
153	B73	1/03	0.96	67.86	69.32	0.75	1.75	1413.20	9.68	0.23	13.31	3.06
157	Tx303	45/03	0.94	73.32	77.04	0.97	1.81	694.64	25.02	0.33	12.61	2.82
	Mean		0.91	68.65	70.56	0.75	1.71	1050.49	11.76	0.23	13.88	3.15
	LSD (0.05)		0.16	2.23	2.61	0.07	0.09	398.28	3.26	0.02	1.98	0.50

TABLE A7. Least square means for single and double-introgression near-isogenic lines from set 7 evaluated as inbreds, *per se,* in 6 North Carolina environments.

dNIL	Line	Pedigree	Seed Source	Stand	Days to Tassel	Days to Silk	Ear Height	Plant Height	Grain Yield	Grain Moisture	Kernel	Protein	Oil
				%	d	d	m	m	g	%	g	%	%
16		TBBC3-02x71	204/03	0.94	66.92	69.82	0.77	1.76	1231.40	11.43	0.24		
17		(TBBC3-02x81)	1355/02	0.65	68.02	71.19	0.71	1.69	706.86	8.54	0.23	15.07	2.70
18		(TBBC3-02x87)	2960/02	0.97	68.76	70.72	0.85	1.85	1390.36	9.70	0.23	13.72	2.67
29		(TBBC3-11x71)	2984/02	0.96	68.10	71.30	0.77	1.73	882.28	14.44	0.20	14.62	2.72
30		(TBBC3-11x81)	1401/02	0.96	69.11	71.56	0.72	1.70	711.15	12.31	0.22	13.76	2.77
31		(TBBC3-11x87)	1330/02	0.86	68.88	70.71	0.71	1.69	1259.59	14.46	0.22	12.91	3.06
46		(TBBC3-13x71)	1331/02	0.87	67.13	68.72	0.71	1.73	964.56	13.17	0.23	14.40	3.18
50		(TBBC3-13x87)	1265/02	0.89	67.97	67.59	0.77	1.76	1552.36	10.28	0.23	12.63	3.05
60		(TBBC3-15x71)	3054/02	0.72	67.87	70.66	0.75	1.74	504.50	17.76	0.24	16.35	2.99
61		(TBBC3-15x81)	3056/02	0.95	68.68	70.79	0.81	1.71	710.18	10.86	0.22	15.40	2.66
62		(TBBC3-15x87)	1321/02	0.68	70.60	71.65	0.80	1.80	1073.84	15.56	0.24	13.74	3.31
71		(TBBC3-16x71)	3172/02	0.97	70.43	71.06	0.82	1.84	1110.65	13.53	0.22	13.63	2.90
72		(TBBC3-16x81)	3210/02	0.87	68.01	69.84	0.72	1.67	1018.69	8.73	0.22	13.67	3.32
137		TBBC3-2	198/02	0.90	70.32	71.91	0.78	1.77	885.39	9.33	0.23	14.29	2.65
138		TBBC3-11	199/02	0.96	69.72	72.61	0.69	1.68	960.23	15.61	0.20	13.10	3.03
139		TBBC3-13	200/02	0.96	68.45	70.09	0.69	1.59	1154.18	9.75	0.23	13.57	3.02

TABLE A7. Continued.

140	TBBC3-15	201/02	0.90	68.87	70.74	0.73	1.67	1056.74	9.41	0.20	14.03	2.95
141	TBBC3-16	124/02	0.96	68.46	69.26	0.77	1.77	1293.75	11.41	0.22	14.19	2.76
150	TBBC3-71	213/02	0.89	68.94	71.17	0.75	1.74	984.82	12.92	0.24	14.73	3.04
151	TBBC3-81	214/02	0.90	67.33	70.17	0.70	1.76	813.54	14.52	0.23	14.80	2.70
152	TBBC3-87	215/02	0.96	67.91	69.25	0.77	1.74	1470.30	11.10	0.22	12.08	3.20
153	B73	1/03	0.76	67.89	69.62	0.73	1.70	1165.19	9.88	0.22	13.32	2.95
157	Tx303	45/03	0.92	75.60	78.47	0.94	1.81	817.57	22.89	0.32	12.75	3.00
161	TBBC3-12	120/02	0.97	67.29	69.79	0.73	1.69	1489.08	14.47	0.22	12.49	3.18
	Mean		0.89	68.80	70.78	0.76	1.73	1050.30	12.59	0.23	13.88	2.95
	LSD (0.05)		0.16	2.05	2.46	0.06	0.07	403.64	3.91	0.02	1.54	0.35

TABLE A8. Least square means for single and double-introgression near-isogenic lines from set 8 evaluated as inbreds, *per se,* in 6 North Carolina environments.

dNIL	Line Pedigree	Seed Source	Stand	Days to Tassel	Days to Silk	Ear Height	Plant Height	Grain Yield	Grain Moisture	Kernel	Protein	Oil
			%	d	d	m	m	g	%	g	%	%
83	(TBBC3-19x71)	1286/02	0.54	69.66	71.29	0.71	1.74	794.55	11.74	0.24	14.85	2.94
84	TBBC3-19x81	5912/03	0.95	68.28	71.32	0.80	1.74	931.27	12.73	0.23		
85	(TBBC3-19x87)	1379/02	0.73	70.23	71.42	0.80	1.77	774.50	12.12	0.22	15.97	3.19
94	(TBBC3-24x71-1)	1300/02	0.95	67.23	69.69	0.78	1.74	1111.23	12.86	0.24	12.49	3.15
95	(TBBC3-24x71-2)	1341/02	0.96	68.12	70.51	0.74	1.60	1184.19	8.80	0.23	12.96	3.05
96	(TBBC3-24x81)	1242/02	0.93	67.82	71.02	0.72	1.69	1101.38	14.50	0.23	13.93	3.16
97	(TBBC3-24x87)	3283/02	0.94	69.56	70.73	0.79	1.72	1049.28	14.36	0.22	13.27	3.19
103	(TBBC3-42x71)	1268/02	0.95	68.31	70.97	0.76	1.80	1241.82	13.47	0.23	13.16	3.21
104	(TBBC3-42x81)	1261/02	0.96	69.82	72.05	0.88	1.89	1332.46	13.22	0.20	13.79	3.10
105	(TBBC3-42x87)	3434/02	0.90	69.79	71.17	0.88	1.97	1262.70	14.98	0.20	12.44	2.86
110	(TBBC3-47x71)	3649/02	0.95	67.20	68.67	0.79	1.77	866.74	12.47	0.25	14.33	3.15
111	(TBBC3-47x81)	1374/02	0.98	68.58	70.29	0.72	1.71	874.20	12.11	0.23	14.95	2.82
112	(TBBC3-47x87)	5864/02	0.96	69.14	70.93	0.79	1.76	1197.88	11.50	0.24	13.83	3.00
117	TBBC3-49x71	214/03	0.89	70.32	71.28	0.79	1.70	961.88	12.25	0.22		
118	(TBBC3-49x81)	1332/02	0.88	69.34	71.14	0.75	1.78	505.25	12.92	0.21	17.31	2.92
119	(TBBC3-49x87)	1377/02	0.93	73.98	73.07	0.83	1.81	808.45	13.81	0.22	13.13	3.05

TABLE A8. Continued.

142	TBBC3-19	203/02	0.95	68.68	70.05	0.76	1.70	1201.38	17.02	0.21	12.82	3.03
143	TBBC3-24	204/02	0.91	67.40	69.55	0.73	1.61	1334.76	13.83	0.21	12.24	3.25
144	TBBC3-42	206/02	0.93	68.27	70.17	0.86	1.88	1446.41	10.08	0.19	12.83	3.21
145	TBBC3-47	207/02	0.86	67.85	68.31	0.74	1.75	855.69	9.64	0.23	13.30	2.80
146	TBBC3-49	208/02	0.92	69.08	69.24	0.81	1.86	694.77	13.78	0.22	15.08	2.98
150	TBBC3-71	213/02	0.88	67.67	70.17	0.72	1.79	858.44	13.41	0.24	14.78	3.11
151	TBBC3-81	214/02	0.96	68.97	70.36	0.76	1.80	991.26	15.63	0.23	14.79	2.85
152	TBBC3-87	215/02	0.89	68.23	68.57	0.83	1.79	1262.62	11.35	0.23	12.37	3.01
153	B73	1/03	0.83	68.21	69.64	0.75	1.72	1220.93	11.53	0.22	13.24	3.00
157	Tx303	45/03	0.97	76.14	78.12	0.95	1.84	684.98	27.08	0.33	13.55	3.01
	Mean		0.90	69.15	70.76	0.79	1.76	1021.12	13.35	0.23	13.81	3.04
	LSD (0.05)		0.11	2.19	2.64	0.07	0.10	383.80	2.80	0.02	1.38	0.44

TABLE A9. Least square means for single and double-introgression near-isogenic lines from set 1 testcrossed to Mo17 evaluated in 7 North Carolina environments.

dNIL Line	Pedigree	Seed Source	Stand	Days to Tassel	Days to Silk	Ear Height	Plant Height	Lodging Root	Lodging Stalk	Erect Plants	Grain Yield	Grain Moisture	Kernel	Protein	Oil
			%	d	d	m	m	%	%	%	Mg ha^{-1}	g kg^{-1}	g	%	%
1	(TBBC3-02x11)xMo17	1451/02	0.95	61.59	64.51	1.02	2.19	0.00	0.10	0.75	6.53	15.23	0.27	10.03	3.49
2	(TBBC3-02x13-1)xMo17	1456/02	1.00	61.55	63.49	1.05	2.19	0.01	0.10	0.75	6.15	14.38	0.29	9.95	3.40
3	(TBBC3-02x13-2)xMo17	1452/02	1.00	61.33	62.95	1.02	2.23	0.02	0.13	0.70	6.33	15.04	0.30	10.46	3.50
4	(TBBC3-02x13-3)xMo17	1455/02	1.00	62.48	64.79	1.05	2.18	0.01	0.12	0.72	6.42	13.97	0.28	11.05	3.58
5	(TBBC3-02x15)xMo17	1459/02	0.94	61.42	63.31	1.02	2.18	0.00	0.08	0.75	6.33	14.62	0.28	10.38	3.44
6	(TBBC3-02x16)xMo17	5591/02	1.00	62.14	63.65	1.10	2.28	0.02	0.14	0.68	6.36	14.45	0.31	9.94	3.35
7	(TBBC3-02x19)xMo17	1460/02	0.96	61.50	63.32	1.04	2.24	0.00	0.10	0.72	6.15	15.06	0.30	9.98	3.43
19	(TBBC3-11x13)xMo17	1476/02	0.98	62.63	63.47	1.00	2.17	0.01	0.11	0.75	6.10	15.23	0.28	10.37	3.30
20	(TBBC3-11x15)xMo17	1479/02	0.96	62.68	63.66	0.99	2.09	0.00	0.09	0.73	5.75	14.70	0.30	11.09	2.76
21	(TBBC3-11x16)xMo17	5599/02	0.98	61.97	63.64	1.07	2.29	0.01	0.07	0.78	6.18	14.89	0.30	10.54	3.28
22	(TBBC3-11x19)xMo17	1480/02	1.00	61.63	63.00	1.01	2.18	0.02	0.08	0.74	5.93	14.70	0.30	10.27	3.25
32	(TBBC3-13x15)xMo17	5618/02	0.99	60.13	63.18	1.00	2.09	0.03	0.10	0.71	6.37	15.06	0.27	9.71	3.68
33	(TBBC3-13x16)xMo17	5619/02	1.00	61.30	62.43	1.06	2.19	0.01	0.08	0.74	6.50	14.91	0.31	10.19	3.36

TABLE A9. Continued.

34	(TBBC3-13x19)xMo17	1495/02	0.99	61.94	63.65	1.04	2.19	0.00	0.06	0.78	6.13	15.26	0.30	10.02	3.33
51	(TBBC3-15x16)xMo17	5622/02	0.98	62.80	64.73	1.14	2.21	0.04	0.10	0.70	6.44	14.62	0.32	10.27	3.48
52	(TBBC3-15x19)xMo17	5623/02	0.99	61.50	63.21	0.99	2.15	0.01	0.11	0.76	5.75	14.24	0.30	9.90	3.28
63	(TBBC3-16x19)xMo17	5642/02	1.00	62.20	63.85	1.02	2.21	0.00	0.04	0.78	6.38	15.09	0.30	10.55	3.39
137	TBBC3-2xMo17	217-222/02	0.96	62.19	63.93	1.06	2.23	0.00	0.10	0.74	6.18	14.47	0.30	10.19	3.63
138	TBBC3-11xMo17	225-230/02	1.00	61.42	64.09	0.98	2.13	0.00	0.10	0.73	6.03	15.63	0.28	9.91	3.55
139	TBBC3-13xMo17	233-238/02	1.00	60.85	63.08	1.00	2.16	0.02	0.11	0.75	6.38	14.92	0.31	10.19	3.58
140	TBBC3-15xMo17	241-246/02	1.00	60.62	62.87	1.01	2.15	0.01	0.07	0.74	6.26	14.80	0.29	9.98	3.44
141	TBBC3-16xMo17	249-254/02	1.00	61.43	62.67	1.07	2.29	0.01	0.07	0.74	6.35	14.75	0.31	10.27	3.32
142	TBBC3-19xMo17	257-262/02	0.98	61.47	62.34	1.02	2.18	0.01	0.09	0.73	6.36	14.95	0.31	10.33	3.24
162	TBBC3-27xMo17	235/03	0.99	61.69	63.25	1.01	2.22	0.01	0.09	0.76	5.94	15.41	0.27	10.43	2.90
153	B73xMo17	57/03	0.95	62.10	63.25	1.03	2.21	0.01	0.09	0.75	6.49	14.83	0.30	10.50	3.24
157	Tx303xMo17	97/03	0.96	65.24	66.69	1.17	2.41	0.03	0.23	0.60	6.30	16.69	0.33	10.84	3.96
	Mean		0.98	61.84	63.58	1.04	2.20	0.01	0.10	0.73	6.23	14.92	0.30	10.28	3.39
	LSD (0.05)		0.04	1.92	1.59	0.05	0.06	0.03	0.08	0.08	0.75	1.04	0.02	0.80	0.40

TABLE A10. Least square means for single and double-introgression near-isogenic lines from set 2 testcrossed to Mo17 evaluated in 7 North Carolina environments.

dNIL	Line	Pedigree	Seed Source	Stand	Days to Tassel	Days to Silk	Ear Height	Plant Height	Lodging Root	Lodging Stalk	Erect Plants	Grain Yield	Grain Moisture	Kernel	Protein	Oil
				%	d	d	m	m	%	%	%	Mg ha^{-1}	g kg^{-1}	g	%	%
8		(TBBC3-02x24)xMo17	5594/02	0.99	60.87	62.63	1.13	2.33	0.06	0.09	0.73	7.01	14.85	0.30	10.47	3.45
9		(TBBC3-02x42-1)xMo17	1464/02	0.96	61.82	62.80	1.08	2.31	0.02	0.07	0.79	6.60	14.55	0.31	10.52	3.14
10		(TBBC3-02x42-2)xMo17	1463/02	1.00	62.76	64.07	1.11	2.28	0.02	0.05	0.79	6.47	15.24	0.28	10.11	3.45
11		(TBBC3-02x47)xMo17	1467/02	1.00	61.76	62.68	1.08	2.27	0.03	0.12	0.72	6.38	14.86	0.31	11.08	3.13
12		(TBBC3-02x49)xMo17	1468/02	0.99	62.59	63.38	1.03	2.27	0.01	0.06	0.81	7.07	14.75	0.30	10.22	3.68
23		(TBBC3-11x24)xMo17	1483/02	1.00	62.37	63.64	1.05	2.22	0.03	0.05	0.78	7.00	15.78	0.30	9.93	3.81
24		(TBBC3-11x42)xMo17	1484/02	1.00	62.40	64.27	1.13	2.32	0.01	0.07	0.76	6.54	15.27	0.29	10.52	2.95
25		(TBBC3-11x49)xMo17	5606/02	1.00	61.48	63.59	1.07	2.33	0.05	0.03	0.76	7.05	15.34	0.30	11.14	2.98
35		(TBBC3-13x24)xMo17	1496/02	1.00	61.79	62.75	1.04	2.20	0.01	0.09	0.78	7.10	15.12	0.31	10.24	3.73
36		(TBBC3-13x24)xMo17	1499/02	1.00	61.70	63.35	1.04	2.26	0.00	0.06	0.82	6.23	15.60	0.30	10.38	3.35
37		(TBBC3-13x24)xMo17	1500/02	1.00	61.29	63.20	1.03	2.20	0.02	0.08	0.76	6.86	15.17	0.30	10.27	3.29
38		(TBBC3-13x42)xMo17	1503/02	0.98	61.63	63.67	1.08	2.29	0.02	0.08	0.76	6.53	15.22	0.30	10.46	3.42
39		(TBBC3-13x47)xMo17	1504/02	0.96	60.87	62.84	1.07	2.25	0.01	0.09	0.77	6.40	14.36	0.29	10.37	3.68
40		(TBBC3-13x49)xMo17	1511/02	0.96	60.63	62.52	1.09	2.28	0.01	0.07	0.76	6.74	14.34	0.32	10.19	3.71
41		(TBBC3-13x49)xMo17	1507/02	0.99	61.29	63.59	1.10	2.31	0.02	0.07	0.77	6.48	14.45	0.30	11.03	3.07

TABLE A10. Continued.

42	(TBBC3-13x49)xMo17	1508/02	1.00	62.02	63.41	1.10	2.29	0.07	0.09	0.71	6.57	14.86	0.32	9.66	3.52
53	(TBBC3-15x24)xMo17	1519/02	1.00	62.10	63.50	1.09	2.25	0.04	0.05	0.75	6.76	15.67	0.29	10.15	3.40
54	(TBBC3-15x42)xMo17	5627/02	0.99	61.36	63.28	1.11	2.23	0.07	0.06	0.72	6.27	15.16	0.27	10.19	3.72
55	(TBBC3-15x47)xMo17	267x268/03	0.97	61.39	63.84	1.03	2.19	0.01	0.05	0.77	6.24	14.17	0.29	10.38	3.39
56	(TBBC3-15x49)xMo17	1528/02	0.97	60.52	64.10	1.07	2.31	0.05	0.06	0.74	6.90	15.18	0.31	9.69	3.72
137	TBBC3-2xMo17	217-222/02	0.99	61.71	63.39	1.07	2.26	0.01	0.06	0.77	6.53	14.17	0.33	10.71	3.53
138	TBBC3-11xMo17	225-230/02	0.99	60.83	63.36	1.06	2.25	0.01	0.07	0.81	6.73	15.37	0.29	9.53	3.38
139	TBBC3-13xMo17	233-238/02	1.00	61.27	63.09	1.06	2.28	0.02	0.05	0.78	6.67	15.16	0.32	10.30	3.35
140	TBBC3-15xMo17	241-246/02	0.99	61.78	63.24	1.05	2.23	0.03	0.07	0.76	6.29	14.78	0.31	10.68	3.44
143	TBBC3-24xMo17	264-271/02	1.00	61.96	63.99	1.04	2.21	0.00	0.04	0.80	7.02	15.15	0.30	9.85	3.43
144	TBBC3-42xMo17	276-283/02	1.00	61.07	62.20	1.15	2.34	0.06	0.09	0.71	6.49	14.81	0.28	10.68	3.33
145	TBBC3-47xMo17	284-291/02	0.97	61.68	62.80	1.09	2.29	0.00	0.10	0.74	6.35	14.67	0.30	10.21	3.42
146	TBBC3-49xMo17	292-299/2	1.00	62.31	64.31	1.09	2.36	0.03	0.06	0.77	7.08	15.24	0.31	10.36	3.36
153	B73xMo17	57/03	0.97	62.36	63.79	1.07	2.29	0.04	0.07	0.75	6.44	14.92	0.30	10.05	3.34
157	Tx303xMo17	97/03	0.97	65.66	67.39	1.18	2.43	0.12	0.17	0.58	6.57	16.25	0.33	10.58	3.62
	Mean		0.99	61.78	63.49	1.08	2.28	0.03	0.07	0.76	6.65	15.02	0.30	10.33	3.43
	LSD (0.05)		0.03	1.59	1.70	0.05	0.05	0.07	0.04	0.08	0.69	0.58	0.02	0.96	0.53

TABLE A11. Least square means for single and double-introgression near-isogenic lines from set 3 testcrossed to Mo17 evaluated in 7 North Carolina environments.

dNIL	Line	Pedigree	Seed Source	Stand	Days to Tassel	Days to Silk	Ear Height	Plant Height	Lodging Root	Lodging Stalk	Erect Plants	Grain Yield	Grain Moisture	Kernel	Protein	Oil
				%	d	d	m	m	%	%	%	Mg ha^{-1}	g kg^{-1}	g	%	%
64		(TBBC3-16x24)xMo17	5643/02	0.99	61.90	63.55	1.08	2.27	0.01	0.04	0.86	6.63	15.74	0.31	10.90	3.41
65		(TBBC3-16x42)xMo17	5646/02	0.97	60.67	62.12	1.12	2.32	0.04	0.10	0.76	6.58	15.48	0.30	10.77	3.11
66		(TBBC3-16x47)xMo17	5647/02	0.98	61.74	63.86	1.14	2.35	0.01	0.07	0.83	6.77	15.51	0.31	11.05	3.39
67		(TBBC3-16x49)xMo17	5650/02	1.00	61.16	63.04	1.02	2.27	0.01	0.04	0.87	6.36	15.32	0.31	10.94	3.04
76		(TBBC3-19x24)xMo17	1535/02	0.98	61.81	63.82	1.05	2.22	0.00	0.03	0.86	6.06	15.93	0.29	10.22	3.46
77		(TBBC3-19x42)xMo17	5785/02x(5750)	0.99	61.40	62.37	1.17	2.32	0.04	0.08	0.79	6.03	15.24	0.30	11.03	2.97
78		(TBBC3-19x47)xMo17	1536/02	0.98	61.20	63.04	1.10	2.29	0.03	0.07	0.81	5.93	15.35	0.29	11.21	2.99
79		(TBBC3-19x49)xMo17	1539/02	1.00	61.78	63.06	1.09	2.29	0.02	0.06	0.81	6.14	15.40	0.31	10.46	3.19
86		(TBBC3-24x42)xMo17	1547/02	0.96	60.86	61.96	1.08	2.22	0.02	0.07	0.80	6.20	15.08	0.30	11.15	3.11
87		(TBBC3-24x47-1)xMo17	5931/03	1.00	59.15	59.54	1.08	2.30	-0.02	0.04	0.89	6.13	15.43	0.30	.	.
88		(TBBC3-24x47-2)xMo17	5934/03	1.00	56.94	59.06	1.07	2.31	-0.01	0.07	0.85	6.34	15.71	0.28	.	.
89		(TBBC3-24x49)xMo17	1548/02	0.98	61.20	63.20	1.05	2.23	0.02	0.04	0.85	6.07	15.93	0.32	10.47	3.14
98		(TBBC3-42x47)xMo17	1564/02	0.99	60.75	62.58	1.12	2.27	0.10	0.09	0.68	6.21	15.30	0.30	11.88	3.16
99		(TBBC3-42x49)xMo17	5671/02	1.00	60.51	62.26	1.09	2.31	0.02	0.07	0.80	6.55	15.79	0.31	10.82	3.03

TABLE A11. Continued.

106	(TBBC3-47x49)xMo17	5850/02x(5832)	0.99	60.62	61.56	1.08	2.29	0.01	0.05	0.83	6.78	15.31	0.31	10.61	3.57
141	TBBC3-16xMo17	249-254/02	0.99	61.38	62.45	1.06	2.31	0.02	0.06	0.80	6.25	15.24	0.32	10.65	3.37
142	TBBC3-19xMo17	257-262/02	0.98	60.78	62.94	1.08	2.29	0.00	0.03	0.88	6.57	15.29	0.30	10.53	3.26
143	TBBC3-24xMo17	265-270/02	0.97	60.93	62.62	1.04	2.24	0.02	0.07	0.82	6.48	15.52	0.30	10.25	3.48
144	TBBC3-42xMo17	277-282/02	1.00	61.22	63.76	1.11	2.28	0.01	0.13	0.75	5.64	15.13	0.29	10.96	3.25
145	TBBC3-47xMo17	285-290/02	1.00	61.04	62.51	1.11	2.31	0.04	0.04	0.82	6.74	14.97	0.31	10.43	3.43
146	TBBC3-49xMo17	293-298/02	1.00	62.15	63.51	1.10	2.35	0.04	0.06	0.81	6.55	15.53	0.32	11.05	3.29
163	TBBC3-54xMo17	236/03	0.99	61.53	62.99	1.10	2.37	0.04	0.07	0.75	6.49	15.76	0.31	11.47	2.40
153	B73xMo17	57/03	0.96	61.18	62.48	1.07	2.28	0.04	0.05	0.81	6.54	15.24	0.31	10.70	3.16
157	Tx303xMo17	97/03	0.95	65.60	67.29	1.21	2.44	0.12	0.15	0.63	6.05	17.43	0.34	11.37	3.58
	Mean		0.98	61.15	62.73	1.09	2.30	0.03	0.07	0.81	6.34	15.53	0.31	10.86	3.22
	LSD		0.05	14.39	13.51	0.05	0.06	0.07	0.07	0.09	0.63	0.73	0.02	1.14	0.34

TABLE A12. Least square means for single and double-introgression near-isogenic lines from set 4 testcrossed to Mo17 evaluated in 8 North Carolina environments.

dNIL Line	Pedigree	Seed Source	Stand	Days to Tassel	Days to Silk	Ear Height	Plant Height	Lodging Root	Lodging Stalk	Erect Plants	Grain Yield	Grain Moisture	Kernel	Protein	Oil
			%	d	d	m	m	%	%	%	Mg ha^{-1}	g kg^{-1}	g	%	%
13	(TBBC3-02x51)xMo17	1471/02	0.99	62.10	64.93	1.06	2.26	0.01	0.14	0.78	6.43	15.30	0.29	10.16	3.60
15	(TBBC3-02x63)xMo17	1472/02	0.98	61.68	63.69	1.06	2.24	0.04	0.18	0.74	6.41	14.46	0.30	10.67	3.12
26	(TBBC3-11x51)xMo17	5607/02	0.97	61.32	63.85	0.98	2.22	0.01	0.09	0.84	6.49	15.23	0.29	10.31	3.36
27	(TBBC3-11x62)xMo17	1488/02	1.00	61.75	63.11	1.06	2.25	0.03	0.08	0.82	6.29	14.90	0.29	10.22	3.20
28	(TBBC3-11x63)xMo17	5610/02	0.94	61.95	62.89	1.10	2.28	0.05	0.09	0.80	7.00	15.00	0.29	10.20	3.40
43	(TBBC3-13x51)xMo17	1512/02	0.98	60.76	62.13	1.05	2.20	0.02	0.13	0.79	5.90	15.00	0.30	10.72	3.70
45	(TBBC3-13x63)xMo17	259/03	0.98	59.35	62.58	1.11	2.35	0.10	0.14	0.70	6.68	15.18	0.32	.	.
58	(TBBC3-15x62)xMo17	5634/02	0.99	62.14	64.51	1.06	2.33	0.07	0.10	0.79	6.24	15.35	0.30	10.63	3.41
59	(TBBC3-15x63)xMo17	1531/02	0.99	61.54	63.21	1.04	2.24	0.07	0.11	0.76	6.20	15.25	0.31	10.22	3.37
68	(TBBC3-16x51)xMo17	5651/02	1.00	61.63	62.32	1.05	2.25	0.05	0.16	0.74	6.42	15.61	0.28	10.54	3.55
69	(TBBC3-16x62)xMo17	5654/02	0.99	61.06	61.83	1.10	2.31	0.03	0.10	0.81	6.54	14.51	0.30	10.32	3.40
70	(TBBC3-16x63)xMo17	5655/02	0.97	61.65	63.17	1.09	2.29	0.07	0.11	0.77	6.40	15.03	0.31	10.35	3.33
137	TBBC3-2xMo17	217-222/02	1.00	61.79	62.96	1.09	2.26	0.03	0.18	0.71	6.53	14.75	0.30	10.15	3.14
138	TBBC3-11xMo17	225-230/02	1.00	61.50	62.85	1.03	2.21	0.02	0.14	0.81	6.46	15.68	0.29	9.70	3.52
139	TBBC3-13xMo17	233-238/02	0.99	61.14	62.16	1.05	2.21	0.02	0.17	0.76	6.17	15.01	0.32	10.52	3.29

TABLE A12. Continued.

140	TBBC3-15xMo17	241-246/02	0.99	61.23	63.00	1.07	2.24	0.07	0.17	0.70	6.25	15.24	0.29	10.07	3.43
141	TBBC3-16xMo17	249-254/02	0.99	61.93	62.69	1.10	2.30	0.06	0.13	0.76	6.58	15.08	0.32	11.17	3.19
147	TBBC3-51xMo17	301-306/02	0.97	61.58	63.09	1.00	2.20	0.03	0.15	0.78	6.16	15.00	0.29	10.14	3.59
148	TBBC3-62xMo17	309-314/02	0.97	60.07	62.59	1.04	2.26	0.02	0.08	0.84	6.43	14.69	0.30	11.00	3.04
149	TBBC3-63xMo17	317-322/02	0.99	61.78	63.65	1.02	2.24	0.02	0.10	0.81	6.11	15.54	0.30	10.93	2.95
153	B73xMo17	57/03	0.96	61.59	62.99	1.06	2.26	0.04	0.12	0.79	6.23	14.86	0.31	10.72	3.14
157	Tx303xMo17	97/03	0.98	65.77	67.33	1.23	2.42	0.12	0.26	0.56	6.28	17.27	0.34	10.78	3.72
	Mean		0.98	61.60	63.25	1.07	2.26	0.05	0.13	0.77	6.37	15.18	0.30	10.45	3.35
	LSD (0.05)		0.03	1.50	1.63	0.04	0.06	0.06	0.09	0.09	0.54	0.65	0.02	0.90	0.35

TABLE A13. Least square means for single and double-introgression near-isogenic lines from set 5 testcrossed to Mo17 evaluated in 8 North Carolina environments.

dNIL	Line	Pedigree	Seed Source	Stand	Days to Tassel	Days to Silk	Ear Height	Plant Height	Lodging Root	Lodging Stalk	Erect Plants	Grain Yield	Grain Moisture	Kernel	Protein	Oil
				%	d	d	m	m	%	%	%	Mg ha^{-1}	g kg^{-1}	g	%	%
80		(TBBC3-19x51)xMo17	5662/02	0.99	61.35	63.12	1.05	2.24	0.02	0.16	0.77	6.28	14.73	0.28	10.11	3.36
81		(TBBC3-19x62)xMo17	5663/02	0.97	62.38	63.49	1.13	2.27	0.01	0.13	0.78	6.45	14.52	0.29	10.65	3.01
82		(TBBC3-19x63)xMo17	1540/02	1.00	60.80	62.69	1.08	2.22	0.02	0.15	0.79	5.92	14.72	0.29	10.83	3.19
90		(TBBC3-24x51)xMo17	1551/02	1.00	60.60	61.96	1.03	2.18	0.01	0.12	0.84	6.23	14.76	0.28	10.88	3.45
91		(TBBC3-24x62)xMo17	1555/02	1.00	61.85	62.38	1.05	2.26	0.01	0.11	0.83	6.32	15.04	0.30	10.71	3.29
92		(TBBC3-24x62)xMo17	1552/02	0.98	61.29	62.30	1.11	2.27	0.02	0.11	0.80	6.36	14.71	0.30	10.37	3.23
93		(TBBC3-24x63)xMo17	1556/02	0.98	61.20	62.22	1.02	2.18	0.02	0.19	0.74	6.20	14.84	0.29	10.41	3.32
100		(TBBC3-42x51)xMo17	1567/02	0.98	61.26	62.37	1.09	2.22	0.01	0.19	0.73	5.84	14.72	0.26	9.92	3.76
101		(TBBC3-42x62)xMo17	1568/02	0.97	61.16	62.20	1.10	2.28	0.02	0.16	0.76	5.88	14.68	0.28	9.87	3.63
102		(TBBC3-42x63)xMo17	1571/02	0.99	62.91	62.84	1.07	2.23	0.02	0.15	0.76	5.94	14.79	0.30	11.19	3.06
107		(TBBC3-47x51)xMo17	1576/02	0.95	60.59	61.56	1.02	2.20	0.05	0.15	0.77	6.41	14.82	0.31	11.17	3.39
108		(TBBC3-47x62)xMo17	5679/02	0.97	59.52	61.81	1.07	2.28	0.04	0.12	0.79	6.34	14.32	0.31	10.27	3.34
109		(TBBC3-47x63)xMo17	5862/02	0.98	61.37	62.78	1.07	2.27	0.03	0.13	0.80	6.35	14.45	0.30	10.41	3.18
113		(TBBC3-49x51-1)xMo17	290/03	0.98	62.50	63.50	1.08	2.25	0.05	0.12	0.78	6.18	14.44	0.30	.	.

TABLE A13. Continued.

114	(TBBC3-49x51-2)xMo17	1583/02	0.98	62.06	62.57	1.13	2.27	0.03	0.09	0.81	6.43	15.24	0.29	9.98	3.47
115	(TBBC3-49x62)xMo17	5683/02	0.98	60.76	62.27	1.12	2.35	0.02	0.13	0.80	6.53	14.67	0.30	10.34	3.30
116	(TBBC3-49x63)xMo17	5875/02x(5901&5902)	1.01	60.39	61.56	1.00	2.22	0.00	0.22	0.77	6.31	15.23	0.30	10.96	3.40
142	TBBC3-19xMo17	257-262/02	1.01	61.11	61.85	1.07	2.25	0.04	0.17	0.74	5.71	14.96	0.29	10.23	3.42
143	TBBC3-24xMo17	265-270/02	0.97	61.20	63.01	1.01	2.20	0.02	0.11	0.82	6.42	14.94	0.29	9.91	3.32
144	TBBC3-42xMo17	277-282/02	1.00	60.25	61.47	1.11	2.28	0.02	0.20	0.74	6.07	15.32	0.27	10.48	3.31
145	TBBC3-47xMo17	285-290/02	0.96	60.41	61.82	1.09	2.28	0.01	0.22	0.70	6.22	14.39	0.28	10.22	3.35
146	TBBC3-49xMo17	293-298/02	0.99	62.00	63.86	1.07	2.29	0.04	0.13	0.78	6.44	15.05	0.31	10.37	3.40
147	TBBC3-51xMo17	301-306/02	0.97	60.07	62.19	1.01	2.16	0.02	0.15	0.77	6.33	14.54	0.29	10.27	3.84
148	TBBC3-62xMo17	309-314/02	0.96	61.11	62.23	1.10	2.26	0.03	0.13	0.76	6.13	15.23	0.29	10.65	3.39
149	TBBC3-63xMo17	317-322/02	0.99	61.33	63.04	1.03	2.20	0.02	0.15	0.80	6.02	15.04	0.30	10.26	3.17
153	B73xMo17	57/03	0.97	61.09	62.53	1.08	2.26	0.02	0.15	0.76	6.30	14.73	0.30	10.67	3.17
157	Tx303xMo17	97/03	0.97	64.49	66.87	1.18	2.39	0.07	0.31	0.59	6.13	16.75	0.32	10.49	3.85
	Mean		0.98	61.30	62.61	1.07	2.25	0.03	0.15	0.77	6.21	14.88	0.29	10.45	3.37
	LSD (0.05)		0.06	1.93	1.67	0.06	0.06	0.04	0.08	0.08	0.53	0.66	0.02	0.93	0.52

TABLE A14. Least square means for single and double-introgression near-isogenic lines from set 6 testcrossed to Mo17 evaluated in 8 North Carolina environments.

dNIL	Line	Pedigree	Seed Source	Stand	Days to Tassel	Days to Silk	Ear Height	Plant Height	Lodging Root	Stalk	Erect Plants	Grain Yield	Grain Moisture	Kernel	Protein	Oil
				%	d	d	m	m	%	%	%	Mg ha^{-1}	g kg^{-1}	g	%	%
120		(TBBC3-51x62)xMo17	5687/02	0.98	61.46	64.02	1.06	2.19	0.11	0.22	0.61	6.15	15.08	0.28	10.07	3.69
121		(TBBC3-51x63)xMo17	5690/02	1.00	62.13	64.27	1.06	2.22	0.07	0.19	0.68	6.34	15.09	0.30	10.60	3.46
122		(TBBC3-51x71-1)xMo17	2907/02	0.97	60.32	62.72	1.06	2.24	0.07	0.15	0.70	6.64	15.08	0.29	10.51	3.71
123		(TBBC3-51x71-2)xMo17	2910/02	0.97	60.60	62.30	1.03	2.24	0.15	0.16	0.68	6.14	15.03	0.31	10.09	3.67
124		(TBBC3-51x81)xMo17	5691/02	0.98	61.19	63.04	1.05	2.19	0.07	0.17	0.71	5.91	15.27	0.28	10.12	3.38
125		(TBBC3-51x87)xMo17	5694/02	1.00	60.52	61.73	1.06	2.22	0.04	0.20	0.69	5.78	14.80	0.28	10.11	3.52
126		(TBBC3-62x63)xMo17	5695/02	1.00	62.33	64.28	1.08	2.27	0.03	0.13	0.81	6.18	14.99	0.30	10.21	3.23
127		(TBBC3-62x71)xMo17	2914/02	0.99	61.00	63.26	1.09	2.32	0.05	0.08	0.79	6.27	14.61	0.31	10.49	3.21
128		(TBBC3-62x81)xMo17	5698/02	0.97	61.43	62.68	1.08	2.27	0.12	0.16	0.64	6.12	15.29	0.30	9.88	3.27
129		(TBBC3-62x87)xMo17	5885/02x(5894&5896)	0.96	61.79	62.27	1.11	2.29	0.09	0.18	0.66	6.33	15.06	0.31	10.35	3.00
130		(TBBC3-63x71-1)xMo17	2915/02	0.99	61.35	63.64	1.04	2.32	0.18	0.15	0.63	6.28	15.09	0.31	.	.
131		(TBBC3-63x71-2)xMo17	298/03	0.97	63.50	64.47	1.07	2.29	0.13	0.14	0.68	6.64	15.65	0.32	10.38	3.62
132		(TBBC3-63x81)xMo17	2919/02	0.95	61.80	63.67	1.03	2.19	0.06	0.17	0.72	5.95	14.94	0.31	10.63	3.25
133		(TBBC3-63x87)xMo17	5699/02	1.00	61.86	62.48	1.11	2.26	0.09	0.22	0.63	6.13	15.36	0.30	9.99	3.46
134		(TBBC3-71x81)xMo17	5702/02	0.99	63.93	64.47	1.15	2.30	0.11	0.21	0.64	6.09	15.88	0.31	10.57	3.46

TABLE A14. Continued.

135	(TBBC3-71x87)xMo17	5703/02	0.99	61.35	62.93	1.06	2.24	0.09	0.13	0.72	6.21	15.66	0.29	10.50	3.60
136	(TBBC3-81x87)xMo17	2922/02	0.95	61.30	61.63	1.07	2.25	0.05	0.23	0.68	5.83	15.26	0.31	10.97	3.04
147	TBBC3-51xMo17	301-306/02	0.99	62.41	63.51	1.04	2.20	0.08	0.21	0.67	5.79	15.83	0.28	10.33	3.53
148	TBBC3-62xMo17	309-314/02	1.00	61.33	63.89	1.08	2.27	0.05	0.12	0.78	6.16	14.80	0.30	10.61	3.10
149	TBBC3-63xMo17	317-322/02	0.98	61.82	63.52	1.00	2.17	0.12	0.14	0.71	6.11	15.61	0.31	10.47	3.20
150	TBBC3-71xMo17	333-338/02	0.99	61.64	65.51	1.06	2.26	0.09	0.11	0.77	6.67	15.31	0.30	10.05	3.97
151	TBBC3-81xMo17	341-346/02	0.98	61.65	62.31	1.07	2.21	0.07	0.22	0.68	6.14	15.60	0.29	9.93	3.57
152	TBBC3-87xMo17	349-354/02	0.97	61.21	62.14	1.07	2.24	0.08	0.19	0.66	5.60	14.77	0.30	10.21	3.37
153	B73xMo17	57/03	0.95	61.64	62.70	1.08	2.26	0.09	0.18	0.68	6.19	15.16	0.30	10.50	3.25
157	Tx303xMo17	97/03	0.95	65.87	68.16	1.17	2.38	0.15	0.35	0.46	6.07	16.92	0.33	11.71	3.69
	Mean		0.98	61.82	63.42	1.07	2.25	0.09	0.18	0.68	6.15	15.29	0.30	10.39	3.43
	LSD (0.05)		0.04	1.79	1.97	0.05	0.05	0.09	0.10	0.12	0.57	0.96	0.02	0.74	0.48

TABLE A15. Least square means for single and double-introgression near-isogenic lines from set 7 testcrossed to Mo17 evaluated in 7 North Carolina environments.

dNIL Line	Pedigree	Seed Source	Stand	Days to Tassel	Days to Silk	Ear Height	Plant Height	Lodging Root	Lodging Stalk	Erect Plants	Grain Yield	Grain Moisture	Kernel	Protein	Oil
			%	d	d	m	m	%	%	%	Mg ha^{-1}	g kg^{-1}	g	%	%
17	(TBBC3-02x81)xMo17	1475/02	0.93	59.96	60.59	1.05	2.21	0.03	0.10	0.78	5.94	14.90	0.30	10.41	3.28
18	(TBBC3-02x87)xMo17	5598/02	0.96	60.15	61.34	1.09	2.22	0.10	0.20	0.62	6.72	15.29	0.30	10.42	3.41
29	(TBBC3-11x71)xMo17	5611/02	0.97	59.84	62.69	1.11	2.30	0.08	0.07	0.74	6.81	15.33	0.28	9.88	3.88
30	(TBBC3-11x81)xMo17	1491/02	0.99	59.76	61.03	1.05	2.20	0.06	0.04	0.83	6.60	15.45	0.31	10.40	3.19
31	(TBBC3-11x87)xMo17	1492/02	0.94	61.36	61.93	1.06	2.24	0.06	0.10	0.75	6.40	15.52	0.28	9.64	3.39
46	(TBBC3-13x71)xMo17	1515/02	1.01	60.23	62.08	1.02	2.21	0.06	0.06	0.80	6.75	15.82	0.28	10.36	3.27
50	(TBBC3-13x87)xMo17	1516/02	0.96	60.18	60.93	1.05	2.25	0.17	0.11	0.65	6.27	15.99	0.29	10.47	3.18
60	(TBBC3-15x71)xMo17	5635/02	0.96	59.44	62.64	1.07	2.27	0.05	0.05	0.82	6.79	14.41	0.28	10.50	3.45
61	(TBBC3-15x81)xMo17	5638/02	0.94	61.34	62.89	1.14	2.22	0.09	0.08	0.74	6.59	15.44	0.29	10.47	3.32
62	(TBBC3-15x87)xMo17	1532/02	1.03	61.78	62.59	1.10	2.25	0.16	0.08	0.71	6.27	14.72	0.29	10.46	3.37
71	(TBBC3-16x71)xMo17	5658/02	1.01	60.97	62.43	1.07	2.28	0.06	0.04	0.79	5.84	15.01	0.29	10.48	3.36
72	(TBBC3-16x81)xMo17	5659/02	0.99	59.41	60.96	1.10	2.23	0.09	0.06	0.78	6.31	15.07	0.31	10.75	3.27
137	TBBC3-2xMo17	217-222/02	0.99	60.71	61.95	1.08	2.25	0.04	0.08	0.78	5.99	15.23	0.30	9.84	3.25
138	TBBC3-11xMo17	225-230/02	0.97	60.32	61.18	1.04	2.18	0.10	0.10	0.74	6.22	15.48	0.26	10.00	3.36
139	TBBC3-13xMo17	233-238/02	0.96	59.99	60.73	1.05	2.21	0.11	0.10	0.71	5.63	15.79	0.29	10.03	3.34

TABLE A15. Continued.

140	TBBC3-15xMo17	241-246/02	0.98	59.94	61.07	1.05	2.19	0.13	0.09	0.66	6.64	15.70	0.30	10.19	3.58
141	TBBC3-16xMo17	249-254/02	1.00	59.80	62.26	1.09	2.30	0.02	0.07	0.80	6.44	14.93	0.31	10.67	3.14
150	TBBC3-71xMo17	333-337/02	1.00	59.76	62.64	1.05	2.23	0.03	0.07	0.81	6.45	14.87	0.30	10.05	3.56
151	TBBC3-81xMo17	341-346/02	0.96	60.05	61.39	1.05	2.21	0.14	0.04	0.75	6.69	15.47	0.30	10.39	3.35
152	TBBC3-87xMo17	349-354/02	0.96	60.22	61.04	1.07	2.22	0.11	0.07	0.74	6.43	14.99	0.29	10.46	3.34
161	TBBC3-12xMo17	223x224/F99	0.98	60.26	62.15	1.05	2.18	0.03	0.05	0.84	6.45	15.29	0.29	10.02	3.43
153	B73xMo17	57/03	0.98	60.21	61.63	1.06	2.23	0.07	0.07	0.79	6.38	15.24	0.30	10.53	3.36
157	Tx303xMo17	97/03	0.84	63.33	65.47	1.15	2.35	0.25	0.19	0.51	6.16	17.10	0.35	11.13	3.67
	Mean		0.97	60.39	61.90	1.07	2.24	0.09	0.08	0.75	6.38	15.35	0.30	10.33	3.38
	LSD (0.05)		0.08	1.52	1.60	0.05	0.06	0.09	0.08	0.11	0.87	0.96	0.03	0.92	0.38

TABLE A16. Least square means for single and double-introgression near-isogenic lines from set 8 testcrossed to Mo17 evaluated in 8 North Carolina environments.

dNIL	Line Pedigree	Seed Source	Stand	Days to Tassel	Days to Silk	Ear Height	Plant Height	Lodging Root	Lodging Stalk	Erect Plants	Grain Yield	Grain Moisture	Kernel Protein	Oil
			%	d	d	m	m	%	%	%	Mg ha^{-1}	g kg^{-1}	g	%	%
83	(TBBC3-19x71)xMo17	1543/02	0.92	60.49	61.61	1.03	2.26	0.12	0.11	0.71	5.92	14.80	0.30	10.24	3.38
84	(TBBC3-19x81)xMo17	5929/03	0.92	61.04	62.07	1.09	2.27	0.04	0.17	0.71	5.78	15.37	0.30	.	.
85	(TBBC3-19x87)xMo17	1544/02	0.98	61.54	62.06	1.11	2.30	0.11	0.14	0.70	6.34	14.67	0.31	10.83	3.01
94	(TBBC3-24x71-1)xMo17	1559/02	0.89	60.95	61.94	1.06	2.25	0.10	0.10	0.75	6.08	15.62	0.31	10.11	3.35
95	(TBBC3-24x71-2)xMo17	1560/02	0.96	60.11	61.86	1.01	2.17	0.03	0.15	0.76	6.54	14.77	0.31	10.64	3.39
96	(TBBC3-24x81)xMo17	1563/02	0.96	59.96	61.18	1.05	2.24	0.16	0.09	0.71	6.24	16.05	0.29	10.77	3.41
97	(TBBC3-24x87)xMo17	5670/02	0.97	60.66	62.70	1.07	2.23	0.15	0.15	0.68	6.30	15.60	0.30	10.33	3.27
103	(TBBC3-42x71)xMo17	1572/02	0.94	59.94	61.91	1.09	2.26	0.13	0.12	0.68	6.32	14.47	0.28	10.88	3.59
104	(TBBC3-42x81)xMo17	1575/02	0.93	60.07	60.87	1.14	2.30	0.19	0.15	0.63	5.41	14.77	0.28	10.62	3.31
105	(TBBC3-42x87)xMo17	5675/02	1.01	60.86	62.53	1.09	2.29	0.09	0.17	0.66	6.56	15.51	0.29	10.24	3.12
110	(TBBC3-47x71)xMo17	5682/02	0.98	60.64	62.28	1.06	2.27	0.14	0.15	0.65	6.86	14.38	0.32	10.84	3.56
111	(TBBC3-47x81)xMo17	1580/02	0.97	61.21	62.25	1.03	2.24	0.09	0.14	0.68	6.09	14.59	0.32	10.96	3.40
112	(TBBC3-47x87)xMo17	5864/02x(5819)	1.01	61.02	61.87	1.06	2.27	0.13	0.17	0.62	6.36	14.99	0.30	10.79	3.24
117	(TBBC3-49x71)xMo17	5935/03	0.95	61.64	63.35	1.17	2.45	0.17	0.09	0.68	6.20	16.14	0.31	.	.

TABLE A16. Continued.

118	(TBBC3-49x81)xMo17	2903/02	0.88	62.20	63.15	1.07	2.31	0.09	0.09	0.70	5.94	14.98	0.30	11.20	3.33
119	(TBBC3-49x87)xMo17	5686/02	0.97	62.15	62.75	1.14	2.31	0.15	0.16	0.65	6.30	14.73	0.28	10.51	3.14
142	TBBC3-19xMo17	257-262/02	0.93	60.38	62.08	1.05	2.22	0.07	0.10	0.79	6.67	14.90	0.29	10.96	3.23
143	TBBC3-24xMo17	265-270/02	0.95	61.05	62.49	1.00	2.16	0.13	0.12	0.68	5.96	15.54	0.30	10.21	3.50
144	TBBC3-42xMo17	277-282/02	0.98	61.03	61.95	1.11	2.29	0.14	0.19	0.60	6.14	15.69	0.25	10.56	3.50
145	TBBC3-47xMo17	285-290/02	0.91	60.67	61.95	1.06	2.25	0.13	0.18	0.60	5.95	15.34	0.30	9.96	3.31
146	TBBC3-49xMo17	293-298/02	0.93	61.03	63.09	1.11	2.35	0.09	0.15	0.72	6.53	15.00	0.30	10.01	3.29
150	TBBC3-71xMo17	333-338/02	0.97	60.81	63.26	1.06	2.28	0.11	0.12	0.73	5.90	15.30	0.30	10.38	3.41
151	TBBC3-81xMo17	341-346/02	0.98	60.46	62.18	1.04	2.18	0.14	0.13	0.65	5.94	15.43	0.30	10.21	3.25
152	TBBC3-87xMo17	349-354/02	0.94	61.32	61.58	1.05	2.24	0.18	0.15	0.62	6.30	15.34	0.29	10.85	3.48
153	B73xMo17	57/03	0.90	60.76	62.19	1.04	2.25	0.10	0.14	0.71	6.05	15.40	0.29	10.58	3.36
157	Tx303xMo17	97/03	0.81	63.79	67.30	1.14	2.35	0.26	0.28	0.41	5.71	17.36	0.31	10.63	3.47
	Mean		0.94	60.99	62.40	1.07	2.27	0.12	0.14	0.67	6.17	15.26	0.30	10.56	3.35
	LSD (0.05)		0.08	1.80	1.76	0.05	0.05	0.10	0.07	0.10	0.79	0.94	0.03	1.21	0.44