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# Molecular marker-facilitated study of quantitative trait loci in a maize population 

Jarboe, Sue Gau, Ph.D.<br>Iowa State University, 1993

# Molecular marker-facilitated study of quantitative trait loci in a maize population 

## by

## Sue Gau Jarboe

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY

Department: Agronomy Major: Plant Breeding

Approved:

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## GENERAL INTRODUCTION <br> Review of Literature

## Application of RFLP Markers in Plant Breeding

Since the suggestion of the potential of Restriction Fragment Length Polymorphisms (RFLPs) as markers in plant breeding (Beckmann and Soller, 1983; Burr et al., 1983), plant breeders, cooperating with plant molecular geneticists, have investigated several possible approaches to utilizing RFLPs. RFLP-based linkage maps have been developed in several plant species (Tanksley, 1989), including maize, (Helentjaris, 1987; Coe et al., 1988; Burr et al., 1988), tomato (Bernatzky et al., 1988; Tanksley et al., 1988), soybean (Apuya et al., 1988; Keim et al., 1989), lettuce (Landry et al., 1987) potato (Bonierbale et al., 1988) and rice (McCouch et al., 1988). Construction of a linkage map provided the basis for further studies.

Linkage between RFLPs and major genes was proposed to be an efficient means of facilitating selection programs with marker assisted screening (Stuber and Edwards, 1987; Tanksley et al., 1989). A very promising aspect of RFLP analysis might be dissection and location of quantitative trait loci (QTL) (Michelmore et al., 1988). Plant geneticists had never seen such a high abundance of genetic markers in the genome until RFLPs were discovered. The identification of QTL would enhance approaches to basic genetics and breeding methodology.

QTL dissection had very important implications in breeding since most traits being improved were quantitative traits controlled by multiple genes. Modern breeding programs have depended on the basic studies of quantitative genetics. Since the proposal of the multiple-factor theory (East, 1916), quantitative genetics has provided valuable information to breeders by using biometrical approaches to characterize traits with pooled
effects of "genetic factors", i.e. genes in modern terms (Mather and Jinks, 1982). In the statistical procedures, phenotypic distributions of variables (measurements of traits) were analyzed and estimates of statistical effects of genes were derived through proposed models. The mathematical models simulated inheritance patterns and assumed all the genetic factors involved in the inheritance for the same traits contributed equally to the variation. While quantitative genetics was still contributing to plant breeding, molecular biology provided new approaches for analysis and manipulation of chromosome regions controlling quantitative traits.

The potential of RFLPs for locating and analyzing quantitative traits has been explored by maize geneticists and breeders. One of the early attempts of QTL dissection was by identifying associations between isozyme markers and QTL (Edwards et al., 1987). Single-factor analysis of variance was used for each pairwise combination of quantitative trait and marker locus. F-test determined if significant variation in trait expression was associated with differences in marker genotypic classes. Significant Ftests were interpreted to indicate segregation of genotypes at a QTL which is linked to the marker locus. For each locus with two alleles, if the cross was between two homozygous lines carrying alternative alleles, three classes would be present in the $F_{2}$ and succeeding $F_{3}$ generation. $A$ significant difference for trait expression among marker genotypic classes indicated an association between the marker loci and QTL for the trait. The minimum number and approximate chromosome regions of genes conditioning a quantitative trait were determined by screening the population with a large number of markers that uniformly covered the genome. The minimum number of genes or chromosome regions containing the genes was represented by the number of the markers closely linked to the QTL. The location of the loci was indicated by the relative position of the marker with other
markers. The gene effects were derived through linear regression (Falconer, 1989) of the trait phenotypes on the genotype classes for each individual locus. Mather and Jinks (1977) elucidated the analysis of QTL components in a factorial fashion with additive and dominance as main effects and their interaction as epistasis. Various models have been proposed with different reference populations, notations and purposes in breeding programs (Hayman, 1958; Hayman, 1960; Gamble, 1962a and b; and Gardner and Eberhart, 1966). All these models were based on the pooled gene effects in the populations. Development of molecular markers has showed the possibility to estimate the effects on the single gene (chromosome region) basis.

Chromosome regions for yield and related traits were identified in maize populations by the association with isozyme markers (Edwards et al., 1987; Stuber and Edwards, 1987; Stuber and Sisco, 1991).

The development of interval mapping provided an alternative approach for QTL location (Lander and Botstein, 1986; 1989). This method was also termed maximum likelihood mapping because the maximum likelihood function was employed to define the probability of a QTL being located on a defined region. LOD score was defined as LOG $_{10}$ [(Odds of the QTL present within the boundary of the two flanking markers]/[Odds of the QTL absent within the boundary)]. This was used as the significance level in interval mapping.

The precision of interval mapping was attributable to the reduced error caused by crossing over between the marker and QTL. The detection procedure was based on linkage disequilibrium between marker loci and QTL. The degree of the disequilibrium was influenced by the recombination frequency. Estimates were most accurate when no crossing over occurs between the marker and QTL (Mather and Jinks, 1982). The procedure of single factor analysis did not impose any limitation on the possibility of crossing over between the marker loci and the QTL; therefore, false linkage
could appear and real linkage was distorted by crossing over. Interval mapping, on the other hand, restricted the interference of crossing over to a very low possibility. Crossing over, if it occurred, happened in a short distance such as an interval defined by two closely linked marker loci with a very low frequency. When the recombination frequency was not zero, all the estimates about gene effects and variation caused by the effects were biased (Mather and Jinks, 1982).
$F_{2}$ populations were the most efficient populations for analysis since linkage disequilibrium was at a maximum. A minimum sample size at a given level of power to detect the $Q T L$ was obtained in a $F_{2}$ population compared to other progeny.

The ability to detect QTL with RFLP markers was influenced by three factors (Lander and Botstein, 1989; Tanksley et al., 1989). First factor was the magnitude of QTL effects and variation contributed by each locus. Only the gene(s) causing sufficient phenotypic variation were detected. Second factor was the size of the population being screened. These two factors were related to each other. The larger the population became, the smaller the effects that could be detected. If a gene had relatively larger effect, it could be detected in a smaller population. The last factor was the recombination frequency between the RFLP marker locus and QTL. As described before, the precision of mapping depended on the number of the markers and how evenly the markers were distributed throughout the genome. More probes and even distribution permitted a more precise location of QTL.

Interval mapping was used in several crop species to locate genes for important agronomic traits. More than 15 QTL controlling fruit traits were identified in an interspecific tomato population in one environment (Paterson et al., 1990). When evaluated across three environments, 29 QTL were detected. Four of the 29 were detected in three environments, 11 in
two environments and the remainder in one environment (Paterson et al., 1991). Genes conditioning plant height were detected in four maize populations (Beavis et al., 1991). Eleven QTL were distributed on eight of the 10 chromosomes. Different locations were detected in each population. Based on the QTL location for yield performance, relationship between heterosis of maize populations and the QTL for yield were analyzed and indicated the QTL identified were significantly related to heterosis for grain yield (Stuber et al., 1992).

Lande and Thompson (1990) pointed out that molecular markers can not replace trait-based methods. Instead, the new methodology should be integrated to obtain the maximum improvement in the economic value of domesticated populations. In this sturiy, a marker-assisted selection (MAS) was proposed to integrate information from molecular markers into breeding programs. Selection indices were constructed combining information on marker loci and phenotypic variation. The efficiency of MAS was derived through simulation. The conclusion was that efficiency was higher for traits with low heritability. Three practical considerations were discussed: 1) The number of molecular loci for detection of QTL should be a few hundred for typical outcrossing species; 2) Sample size to detect QTLs for traits with low heritability should be rather large to detect the additive genetic variance associated with marker loci; and 3) Sampling error in the estimation of relative weights in the selection index. The reduction of efficiency by sampling error would be very small with a sample size of a few hundred to a few thousand. It was possible to achieve a substantial increase in efficiency of artificial selection by constructing the indices with the information of molecular markers, although the scale of this endeavor might exceed the current capacity of most molecular genetic laboratories.

Controlled environment conditions were used to isolate a single

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environmental factor and locate QTL related to the factor. Reiter et al. (1991) conducted research under an artificial low phosphorus condition and identified chromosome regions related to resistance to low phosphorus. Six regions were identified on five chromosomes.

Most of the initial studies of QTL location were conducted using parental lines well diversified to ensure a sufficient polymorphism of markers and large differentiation among progeny. In most maize breeding programs, parental materials were elite lines with similar morphological traits. Abler et al. (1991) detected QTL in a population with parents morphologically more similar than the materials used in previous studies. Sufficient variation existed in the population for QTL. This conclusion was important for breeders because the evidence pointed to the application of molecular markers in breeding practice.

## Resistance to 2ECB and Other Traits Evaluated

European corn borer (ECB) (Ostrinia nubilalis Hübner) is one of the most destructive insect pests in U.S. maize production. The annual economic loss exceeded $\$ 200$ million (Burkhardt et al., 1978). Yield reduction ranged from 11\% to $34 \%$ in 12 single crosses at different levels of infestation (Guthrie et al., 1975). Development of resistant hybrids has been the most economic and effective way to reduce the grain yield loss (Jarvis et al., 1983).

Information on the genetics of host-plant resistance has been essential to breeding programs. Although several crop species can support ECB, maize is the preferred host. ECB typically has two generations in Central U.S. Corn Belt coinciding with the two growth stages of maize plant development (Dicke, 1954). ECB hibernates as mature larvae in maize stalks or plant debris in a suspended physiological condition of diapause. From May to July, moths emerge after pupation. First generation European corn
borer (1ECB) larvae infest plants in June to early July when plants are in the midwhorl stage. Eggs are laid on the underside of corn leaves in masses of 15 to 30 (Showers et al., 1980). The young larvae (first to fourth instars) feed predominantly on leaves in the whorl and cause reduction of photosynthetic area in susceptible genotypes. The sheath becomes the principle feeding point for the fifth instar larvae and a small number of fifth instar larvae may burrow into stalks. Host plant resistance to $1 E C B$ is expressed as resistance to leaf feeding through antibiosis (Guthrie et al., 1960).

Second generation European corn borer (2ECB) infest corn plants during late July to early September when the plants have reached their reproductive stage (Dicke, 1954). Oviposition of 2ECB is mostly on the underside of the ear leaf and the leaves two above and below primary ears (more than 85\%) (Dicke, 1954). First and second instar larvae primarily feed on pollen accumulation at the axil of leaves and on sheath-collar, ear shoots and silk tissue (Guthrie et al, 1960; 1970). First through fourth instar larvae can develop on a diet of pure pollen, but 75\% of the fourth instar larvae feed extensively on sheath-collar tissue (Guthrie, 1970). Fifth instar larvae tunnel into the stalks and shanks causing direct harvest loss due to broken stalks and dropped ears and indirect loss due to stalk rot, smut and other diseases.

Resistance to 2ECB is chiefly sheath-collar feeding resistance expressed as antibiosis. More than 95\% of 2ECB mortality occurs within three days after eggs hatch on the resistant genotypes (Guthrie et al., 1970). A certain degree of tolerance can be expected in some resistant genotypes with strong stalks; however, sheath-collar rating has been a generally accepted measurement in breeding programs (Guthrie, 1987a).

Estimates of yield losses due to ECB have varied with the methods of access, materials (maize populations) evaluated and other factors. In most
studies of host plant resistance, artificial infestation has been used to ensure uniform and repeatable insect populations. Artificial infestation has contributed tremendously to the success of the breeding programs for resistance to ECB.

Yield reduction caused by 1 ECB has been attributed primarily to the loss of leaf area due to larval feeding. The yield loss by 2ECB can be caused by leaf area reduction and damage to stalk and shank.

The estimates for grain yield loss attributed to 1ECB and 2ECB were obtained with different methods in various genetic backgrounds. Penny and Dicke (1959) compared yield reduction of several types of hybrids under artificial infestation for 1ECB with four egg masses per plant. Twenty percent reduction was found in hybrids of two resistant parents, while yield reduction of $35 \%$ and $60 \%$ were observed in hybrids of resistant $X$ susceptible and susceptible $X$ susceptible crosses, respectively. Scott et al. (1967) found a $12 \%$ yield loss in susceptible $X$ susceptible crosses and $4 \%$ loss in resistant $X$ resistant crosses when infesting with 2ECB of 3 egg masses per plant. Guthrie et al. (1975) infested 12 hybrids with four levels of 2ECB. The average yield reduction of a susceptible hybrid was 40\%. The average loss for 12 hybrids was 23.5\%.

Some early studies indicated that 1ECB caused more damage (Jarvis et al., 1961). Recent studies, however, identified 2ECB as the most damaging generation (Guthrie, 1987b; Lynch, 1980; Duvick, 1984). The discrepancy was explained as a gradual improvement of host-plant resistance to 1ECB. Progress on improving resistance to 2ECB has been relatively slow.

Jarvis et al. (1983) demonstrated that an intermediate level of resistance to 2ECB was sufficient to prevent economic losses. Showers et al. (1983) reported that most modern hybrids were susceptible with various degrees of susceptibility. Duvick (1984) compared hybrids of different eras released by Pioneer Hi-bred International from 1930s through 1970s.

Resistance to 1ECB had been increased significantly over time. Resistance to 2ECB had not shown significant improvement. Reduction of yield losses by 2ECB was primarily derived from increased tolerance attributed to the greater ability of modern corn hybrids to withstand stress and physical change. This phenomena was demonstrated by an earlier study of inbreds and hybrids selected for resistance to stalk-rotting organisms also remained upright despite feeding damage (Hallauer et al, 1988).

## Screening Programs for Resistant Genotypes

Effective screening procedures have been essential for successful breeding programs for resistance. Initially, measurements were taken of the number of established borers in the plants (Patch et al., 1941). This method was not replaced until the 1960's. Cavity counts were reported to be a better estimate (Jarvis et al., 1961). Pesho et al. (1965) first dissected the plants and directly measured the borer damage of 2ECB by splitting stalks.

Successful artificial rearing techniques allowed uniform and controllable level of infestation of breeding materials. Standard methods have been developed after 35 years of breeding practice (Guthrie and Berry, 1987). For evaluation of resistance to 1ECB, maize plants were infested during the midwhorl stage. Infestation level was dependent on the objectives of the research. Germplasm screening has used two applications (about 50 borers per application) spaced approximately three days apart on 6-10 plants per plot. Four applications approximately two days apart were adequate for most studies. At least eight applications should be used for genetic studies to minimize the chance of host-plant escape. Measurements were recorded about six weeks after infestation.

Artificial infestation for 2ECB was applied during anthesis since increased survival of 2ECB larvae was associated with anthesis (Guthrie et
al., 1971). Two or three applications of approximately 50 eggs or larvae per application was the common dosage in breeding programs. Field evaluation of borer damage was conducted 50-60 days after infestation. Measurement of the length of the 2ECB tunnelling in the stalks was an effective method, but not very efficient because the labor and time required for the procedure limited the amount of material that could be evaluated. However, accurate estimates could be obtained for genetic studies, such as locating the chromosome regions containing genes for resistance.

Lesion counts on sheath-collar tissue were proven an efficient and effective method with high correlation ( $r=0.78$ ) to cavity counting (Guthrie et al., 1978). A 9-class grading system was used with class 1 as no injury to sheath-collar, no visible holes in stalks, no visible frass, and class 9 as 76-100\% sheath-collar damage, numerous holes in the sheath visible and abundant frass. This approach has been most extensively used in breeding programs.

## Genetics of Host-Plant Resistance

In breeding programs, when the sources of resistance were identified, the genetics of resistance needed to be determined (Guthrie, 1987). Detailed breeding plans should not be completed until some information has been obtained on the genetics of host-plant resistance.

The studies on the genetic basis of resistance were conducted under relatively high infestation levels to minimize host-plant escape (Guthrie, 1987). Previous information indicated several genetic factors (multiple genes) were involved in the resistance of inbred lines (Jennings et al., 1974a). Thus, resistance was a quantitative trait.

Segregation in an $F_{2}$ population and the backcross populations of M14 (Susceptible) X MSI (resistant) indicated at least three factors were
involved in the inheritance of resistance to 1ECB. Partial dominance of susceptibility was detected (Penny and Dicke, 1959). One or two gene pairs were reported from the cross B14 (susceptible) X N32 (resistant) in the segregation of individual $F_{2}$ plants and backcrosses. A specific stock $g l_{7} V_{17}$ resistant and homozygous for two very closely linked genes, was crossed with WF9, a susceptible inbred. One single resistance gene was identified in the stock, linked with $\mathrm{gl}_{7} \mathrm{v}_{17}$ genes. The crossing over was from 31 to 37\%.

Reciprocal translocations were used to locate genes affecting resistance. The genetic basis of locating genes by translocations was interpreted by Anderson (1956). Segregation of chromosomes affected by the translocation caused unbalanced distribution of genetic material in reproductive cells and resulted in semisterility of plants heterozygous for the translocation. Therefore, semisterility can be used as a phenotypic marker to identify the plants containing a translocation. When the susceptible gene(s) were present on the translocated chromosome, there would be an association between the semisterility and susceptibility, i.e. they would not segregate independently. A set of translocation stocks of maize with translocations for each of the 20 chromosome arms allowed researchers to locate the genes for traits.

Resistant genes for 1 ECB were located on chromosome arms 3L, 4L and 5L in inbred A411 (Ibrahim, 1954), but several chromosome arms were not tested, including $15,4 \mathrm{~S}, 5 \mathrm{~S}, 6 \mathrm{~S}$ and 8 S . Scott et al. (1966) conducted a study with 23 reciprocal translocations covering all 20 chromosome arms. Resistant inbreds CI31A and B49 were crossed to translocation stocks and semisterile $F_{1}$ plants were crossed to susceptible inbreds M14 and WF9. A significant difference between means of semisterile and normal plants indicated a gene(s) for resistance in the resistant inbred on the chromosome arm involved in the translocation. Resistance genes were
located on chromosomes 4S, 6S, $1 \mathrm{~L}, 2 \mathrm{~L}$ and 4 L for inbred CI31A. For inbred B49, resistant genes appeared on all the chromosome arms identified in CI31A plus an additional gene(s) on 8L.

Translocations were used to locate genes for resistance to 2ECB in B52 (Onukagu et al., 1978). The genes were linked to chromosome arms 1L, 2L, 4L, 8L, 1S, 3S and 5S. The limitations of translocation studies were summarized as (Scott et al., 1967; Guthrie, 1987): 1) Linked genes might be identified as a single gene, leading to underestimation of the number of genes; 2) Recessive genes for resistance were not detected; and 3) The effects of a gene had to be detectable in the heterozygous condition.

Some classical quantitative genetic studies have been conducted to describe the features of germplasm and breeding populations for resistance to ECB. Heritability based on 300 S 1 progeny means was estimated as 69.6\% for resistance to 2ECB in Synthetic BS9 (Russell, 1972). Generation means analysis (Hayman, 1958) used $F_{2}, F_{3}$ and selfed backcross populations from the cross CI31A (resistant) X B27 (susceptible) to estimate the gene action for resistance to 1ECB (Scott, 1966). Most genetic variation was attributed to additive gene effects. The inheritance of resistance genes to lecb did not seem very complicated. Another generation means analysis was done to estimate gene action for resistance to 2ECB (sheath-collar feeding). Nine populations ( $P_{1}, P_{2}, F_{1}, F_{2}, F_{3}, B C_{1}, B C_{2}, B S_{1}$ and $B C_{2}$ ) were produced from four crosses with B52 as the common resistant parent in each population (Jennings et al., 1974a). The other parents for the four crosses were B39, L289, OH43 and WF9. Complex gene action was detected with additive gene effects predominant and dominant effects significant in all crosses except for the cross B39 X B52. Resistance to 2ECB might be the cumulative effects of an unknown number of loci with a more complex pattern than that for resistance to 1ECB. Jennings et al. (1974b) evaluated a 10-line diallel for resistance to 2ECB and another 10-line
diallel for resistance to both 1ECB and 2ECB. The results indicated an additive gene action pattern. General combining ability (GCA) and specific combining ability (SCA) were significant in the second diallel and partial dominance was detected. Overall, inheritance of resistance to 2ECB was more complicated than that of 1ECB. Gene action varied among genetic backgrounds.

## Breeding Programs for Resistance and Success

Breeding programs are usually determined by two factors: 1)mode of reproduction and 2 ) gene action involved in the trait(s). Since the discovery of ECB in U.S. early this century, U.S. corn production has gone through a transition from open-pollinated varieties to single-cross hybrids. This change greatly stimulated the search for resistant lines that might be used directly for hybrid combination or breeding programs.

Breeding for resistance to IECB has been more effective and successful than that for 2ECB. Several factors might explain the slower progress in breeding for resistance to 2ECB: 1) Few sources of germplasm with an adequate level of resistance; 2)Poor agronomic performance of the resistant germplasm; 3) Labor-intensive screening procedures did not allow large scale evaluation; 4) Post-anthesis screening and selection coinciding with grain harvest reduced the efficiency of selection; and 5) Possibly, more complex genetic control was involved.

Resistance to 1ECB and 2ECB was governed by different genetic mechanisms: 1) Inbreds resistant to $1 E C B$ might not be resistant to $2 E C B$ (Guthrie, 1987); 2) One of the chemical substances (DIMBOA, 2,4-dihydroxy-methoxy- $2 \mathrm{H}-1,4$-benxozin-3(4H)-one] responsible for resistance to 1 ECB in some cases did not exist when plant reached reproductive stage and exposed to 2ECB infestation (Klun et al., 1970); and 3) Reciprocal translocations located resistance genes for $1 E C B$ and $2 E C B$ on different chromosome arms.

Therefore, breeding programs need to consider them as two traits. Recurrent Selection

The primary objective of a recurrent selection program is to improve the mean performance of the population by increasing the frequency of the favorable alleles while maintaining the genetic variability for the quantitative trait (Hallauer and Miranda, 1988). Recurrent selection is a good alternative when backcross, pedigree and phenotypic selection are not effective for improving quantitative traits.

Penny et al. (1967) conducted three cycles of $S 1$ recurrent selection in five synthetic populations for resistance to 1ECB. In five populations, only four out of 300 (1.3\%) S1 lines were rated resistant in $C 0$ populations. Two cycles of gelection increased the frequency of resistant lines to 50\%. Sixty five percent of the lines were rated resistant after the third cycle. Recurrent selection was effective for increasing the level of resistance to 1ECB.

The same five populations were reevaluated for correlated changes for 11 plant, ear and grain traits (Russell and Guthrie, 1979). Comparisons were made between C3 and CO in testcrosses in noninfested plots. The increased resistance to 1 ECB was accompanied by correlated negative changes in agronomic traits possibly due to inbreeding depression and/or changes of gene frequencies in the selection process.

Recurrent selection was successful in Synthetic BS1 to improve resistance to 1ECB by selection for increased DIMBOA concentration (Tseng et al., 1984). Nineteen percent of the 51 lines in BS1CO were rated resistant. Selection was conducted using two criteria, DIMBOA concentration and field evaluation for resistance. When selected on the basis of field performance, 75\% of the lines were ranked resistant in the C3. With selection on the basis of DIMBOA concentration, $95 \%$ of the lines in the $C 3$ were ranked resistant.

Resistance to 2ECB has become a mure important concern to breeders because of the relatively heavier economic losses in recent years. Synthetic BS9 was developed as a source for resistance to 1ECB and 2ECB. The 10 component inbred lines varied in their resistance to the two generations and were selected on the basis of their combining ability for grain yield. The data concerning resistance for the 10 lines were obtained from a study by Pesho et al.(1965). Three hundred SI lines were evaluated and $10 \%$ were selected and recombined to form the next cycle. Release of BS9(CB)C4 to hybrid seed industry (Russell and Guthrie, 1982) was a significant event because it was the first Corn Belt synthetic specifically developed for resistance to ECB for the whole life of the corn plant. Several other synthetic populations were released for resistance to both 1ECB and 2ECB, including synthetic populations BS17(CB)C4 and BS16(CB)C4 (Russell and Guthrie, 1991).

The effects of recurrent selection in BS9 were examined (Klenke et al., 1986) in the base population (CO) and four succeeding cycles of selection. Resistance and agronomic traits were evaluated. Significant increases for resistance were found from BS9C0 to BS9C4 (leaf feeding, sheath-collar feeding and stalk tunnelling) in average 51 means, population per se and population testcrosses; however, significant reduction of grain yield occurred as a correlated response from selection for resistance. Klenke et al. (1987) analyzed the cause of the grain yield reduction by the Smith model (Smith, 1979) and concluded that inbreeding depression was an important factor affecting performance of advanced cycles of selection. Inbreeding depression resulted from the random fixation of alleles at the loci which were heterozygous in the original population. , i.e., genetic drift. Unfavorable linkage might be another important cause for indirect negative yield response to selection for resistance.

## Pedigree Selection

Pedigree gelection has been one of the most widely used breeding strategies employed in inbred line development. Inbred B86 was developed to combine the resistance to 1 ECB from Oh43 and resistance to 2 ECB from B52 (Russell et al., 1974). $F_{2}$ through $F_{6}$ populations derived from cross Oh43 $X$ B52 were infested with two generations of ECB. Only the lines with adequate resistance to both generations were advanced to the next generation.

Grain yield is the primary trait in most breeding programs (Hallauer et al., 1988). As grain yield is a complex trait, study of the component traits will provide related information for grain yield improvement. Breeders also ranked maturity and plant stature as important traits in breeding programs (Bauman, 1980; Hallauer, 1981).

In this study, three experiments were conducted using RFLPs as a tool to analyze the chromosome regions controlling the inheritance of traits. The objectives are:

1. to locate and analyze the chromosome regions conferring resistance to 2ECB in hill plots and compare the results from different environments. 2. to locate and analyze the chromosome regions conferring maturity and plant stature in single row plots and compare the results in different environments.
2. to locate and analyze the chromosome regions conferring grain yield and component traits and compare the results from different environments.

## Population Development

The population used for RFLP linkage map development and field evaluation was derived from crossing inbred lines Mol7 and B52. Mol7 is an inbred representing Lancaster Sure Crop. It has been extensively used in commercial hybrid production and breeding programs because of its good
combining ability and high heterotic expression when crossed with inbred lines from Reid Yellow Dent (Hallauer et al., 1988). It has desirable agronomic traits, but it is highly susceptible to feeding damage by 2ECB.

B52 is adapted to the central U.S. Corn Belt, but has not been used in production or breeding programs very much due to its low combining ability in $F_{1}$ hybrids and undesirable agronomic traits (Guthrie, 1987). B52 was released in 1959 as a source of resistance to 2ECB (Pesho and Dicke, 1961). The $F_{2}$ population was obtained by selfing the $F_{1}(B 52 \times$ Mol7) hybrid. An unselected sample of $150 \mathrm{~F}_{2}$ plants was selfed-pollinated to produce an $\mathrm{F}_{3}$ family. For each $F_{2: 3}$ family, 15 kernels were planted in the greenhouse to obtain leaf tissue for DNA isolation in RFLP analysis, and the remnant seeds were used for field evaluation over environments.

The same $150 \mathrm{~F}_{3}$ lines used in RFLP analysis were evaluated for resistance to 2 ECB and several agronomic traits. A $12 \times 13$ rectangular Lattice Design included $150 \mathrm{~F}_{3}$ lines and six checks of two plots for each of Mol7, B52 and $F_{1}$.

## Explanation of Dissertation Format

The three papers of this dissertation describe different experiments conducted to map genes for resistance to $2 E C B$, morphological traits and yield component traits, respectively. All experiments were part of the dissertation research.

Paper I includes an experiment in which the primary trait was resistance to 2ECB evaluated in hill plots. Three other morphological traits were also evaluated. Paper II included an experiment in which three morphological traits were studied in single row plots. Paper III analyzed eight traits which were considered as grain yield components. The three papers of the dissertation are preceded by a review of the literature in the General Introduction and followed by a General Summary of the entire
dissertation. References cited in the General Introduction are listed in the General References following the General Summary. Appendices include the data not directly included in the discussion, but provide some insight for interested readers.

# PAPER I. LOCATION AND ANALYSIS OF CHROMOSOME REGIONS AFFECTING RESISTANCE TO SECOND GENERATION EUROPEAN CORN BORER AND THREE MORPHOLOGICAL TRAITS IN A MAIZE POPULATION 

## ABSTRACT

European corn borer (Ostrinia nubilalis Hübner, ECB) has been one of the most destructive insect pests in U.S. maize production. Utilization of resistant hybrids has been the most economic and successful means of reducing the grain yield losses.

In this study, a segregating population was created by crossing two inbred lines, 852 and Mol7, and self-pollinating the $F_{1}$ hybrid. RFLPs were used to locate and analyze the chromosome regions affecting resistance to second generation European corn borer (2ECB) and three agronomic traits, including plant height, ear height and flowering date [measured as anthesis and silk emergence by growing degree days (GDD)] in the population. One hundred and fifty $F_{2: 3}$ lines were evaluated for the traits and analyzed for their RFLP phenotypes. One hundred and thirteen genomic and cDNA clones were included to construct a maize linkage map based on which QTL were detected over the genome.

Putative QTL for 2ECB were located on chromosomes 1, 2, 3, 4, 7, 8, 9 and 10. QTL on chromosomes $1,2,9$ and 10 were detected in more than one environment. QTL on chromosomes 1,2 and 9 seemed to be more important than other regions. Most regions contributing to increased resistance were derived from B52. All chromosomes detected by previous translocation mapping were identified by RFLP mapping.

QTL for plant and ear height were located on chromosomes 1 and 8 in all environments. Also, QTL for silk emergence were located on chromosomes 1 and 8 in all environments. QTL for highly correlated traits were often detected in the same regions of the linkage map.

## INTRODUCTION

RFLP Markers in Plant Breeding
Restriction Fragment Length Polymorphism (RFLP) markers have been proposed to be of great potential in plant breeding (Beckmann et al., 1983). Plant breeders, cooperating with plant molecular geneticists, have investigated several possible approaches to utilizing RFLPs in plant breeding. One of the most promising application of RFLP marker may be dissection and location of individual genes or chromosomal segments controlling quantitative traits (Lander and Botatein., 1989; Edwards et al., 1987).

Quantitative trait loci (QTL) for 25 agronomic traits were mapped in maize populations by isozyme markers(Edwards et al., 1987). The QTL appeared to be distributed throughout the genome. Each region accounted for varying proportion of the phenotypic variation. Gene action varied among regions. The authors pointed out that dominance and overdominance might be pseudo-overestimated due to linkage disequilibrium population in the. The conclusion from their study was that molecular markers could be used to identify QTL for traits important for breeding. Edwards et al. (1992) saturated the genome with RFLP marker to get better perspective of the genome for QTL location. The regions identified for yield component traits corresponded well with the previous study. gTL for grain yield and 24 component traits were identified in two maize populations by isozyme markers (Stuber and Edwards, 1987). Regions identified for grain yield components contributed different amounts of variation and exhibited different gene action.

Eleven QTL for plant height were located in four maize populations (Beavis et al.; 1991). The QTL appeared to be associated with qualitative genetic loci. They indicated that the current methods of QTL location
(linear regression and interval mapping) probably identified sites in the maize genome that maize geneticists have identified for pant height.

Putative QTL for tolerance to low-phosphorus were located on five regions of four chromosomes (Reiter et al., 1991). One region contained two linked markers with significant additive by additive interaction. Four other regions functioned independently with major additive gene action.

Isozymes were used to locate $Q T L$ in six $F_{2}$ populations. The results indicated that elite lines used as parents in breeding programs have adequate quantitative trait variation and QTL can be detected (Abler et al., 1991). The impact of these results was important because the experimental material was closer to that used in breeding programs than the former studies where selection of parents focused on the maximum polymorphism between parental lines.

## Resistance to Second Generation European Corn Borer

European corn borer (Ostrinia nubilalis Hübner, ECB) is one of the most destructive insect pests in U.S. sorn production with annual economic loss exceeding $\$ 200$ million (Burkhardt et al., 1978). Grain yield reduction from $11 \%$ to $34 \%$ in 12 single cross hybrids has been reported (Guthrie et al., 1975). Development of resistant hybrids has been the most economic and effective way to reduce the grain yield loss (Jarvis et al., 1983).

In U.S. Central Corn Belt, ECB typically has two generations coinciding with two growth stages of corn plant development (Dicke, 1954). ECB hibernate as mature larvae in diapause in corn stalks. From May to July, moths emerge after pupation and oviposit on plants in the vegetative stage.

Second generation European corn borer (2ECB) moths infest maize fields from late July to early September when the plants have reached the
reproductive stage (Dicke, 1954). Oviposition of 2ECB moths is mostly on the underside of the ear leaf and two leaves above and below the primary ear. Firgt and second instar larvae primarily feed on pollen accumulation at the leaf axil, sheath-collar, ear shoots and silk tissue (Guthrie, 1960 and 1979). First through fourth instar larvae can develop on a diet of pure pollen, but most (75\%) of the fourth instar larvae fed extensively on sheath-collar tissue (Guthrie, 1970). Fifth instar larvae tunnel into the stalks and shanks, causing direct harvest loss due to broken stalks and dropped ears and indirect loss due to stalk rot, smut and other diseases.

Estimates of grain yield loss have been obtained with different methods in various genetic backgrounds. Scott et al. (1967) found 12\% yield losses in susceptible $x$ susceptible crosses and $4 \%$ in resistant $x$ resistant single cross hybrids when infested with 2ECB at a rate of three egg masses per plant. Guthrie et al. (1975) infested 12 hybrids with 2ECB at different levels: $4,8,12,16$ and 20 egg masses per plant. The extremely susceptible hybrids had an average grain yield reduction of $40 \%$. The average grain yield reduction for the 12 hybrids was 23.5\%.

Recent studies have identified 2ECB as the most damaging generation (Lynch, 1980; Duvick, 1984; Guthrie, 1987). Jarvis et al. (1983; 1991) demonstrated that an intermediate level of resistance to 2ECB may be sufficient to prevent losses of economic significance. Showers et al. (1983) reported that most modern hybrids were susceptible with various degrees of susceptibility. Duvick (1984) compared hybrids released by Pioneer Hi-Bred International from 1930's through 1970's. Resistance to 2ECB did not show significant improvement; however, modern hybrids exhibited less reduction of grain yield under 2ECB infestation. Reduction of grain yield losses by 2ECB was primarily attributed to increased tolerance of modern corn hybrids to withstand stress in general. In other reports, inbreds and hybrids with high levels of resistance to stalk-
rotting organisms had less stalk breakage and fewer dropped ears after 2ECB infestation (Hallauer et al., 1988). Improvement of root, stalk and shank strength has contributed to hybrids' tolerance to 2ECB to a large extent (Guthrie et al. 1979).

## Screening Procedures for Resistant Genotypes

Since successful artificial rearing of corn borer has been achieved, artificial infestation has been common practice in screening for genotypes resistant to 2ECB (Guthrie, 1987). Two methods were standardized for recording 2ECB damage. Measurement of 2ECB tunnelling in stalks (Pesho et al. 1965) was an effective method, but it required much labor and time. Lesion counts on sheath-collar tissue has been proven to be an efficient and effective method with high correlation ( $r=0.78$ ) to 2ECB damage in stalks (Guthrie et al., 1978). The rating system consisted of a nine-class scale with class 1 as no injury to sheath-collar, no visible holes in stalks, no visible frass, and class 9 as 76-100\% sheath-collar damage, numerous holes in the sheath and much frass. This approach has been used most extensively in breeding programs since its high efficiency and fairly good selection response.

Resistance to 2ECB was chiefly sheath-collar feeding resistance and expressed as antibiosis. More than $95 \%$ of 2 ECB mortality occurred within three days after eggs hatched on resistant genotypes (Guthrie et al., 1970).

Genetics of Host-Plant Resistance
Studies on the genetic basis of resistance have been done under relatively high infestation level to minimize host-plant escape (Guthrie, 1987). Several genetic factors determined resistance of inbred lines (Jennings et al., 1974a and b). Thus, resistance was considered a
quantitative trait.
Genetic factors determining resistance to 1ECB and 2ECB were different in several regards: 1) Inbreds resistant to 1 ECB might not be resistant to 2ECB (East, 1916); 2) One of the chemical substances [DIMBOA, 2,4-dihydroxy-methoxy-2H-1,4-benxozin-3(4H)-one] determined to be a source of resistance to $1 E C B$ in some cases was nearly absent when the plant reached the reproductive stage (Klun et al., 1970); and 3) Reciprocal translocations located resistant genes for 1 ECB and 2ECB on different chromosome arms.

Reciprocal A-A translocations were used to locate genes for resiatance to 2ECB in inbred B52 (Onukagu et al., 1978). Chromosome arms 1L, 1S, 2L, 3S, 4L, 5S and 8L were identified. The limitations of translocation mapping were noted by Scott et al. (1966) and Guthrie (1987): 1) Linked genes were identified as a single gene and led to an underestimate of the number of genes; 2) Recessive genes for resistance were not detected; 3) Only genes expressing a detectable level in the heterozygous condition could be detected; and 4) The amount of the genome linked to the breakpoints influenced the ability to detect the gene(s).

Jennings et al. (1974a) conducted generation means analyses in four crosses with B52 as the common resistant parent to estimate gene action for resistance to 2ECB (sheath-collar feeding). Nine populations ( $P_{1}, P_{2}, F_{1}$, $F_{2}, F_{3}, B C_{1}, B C_{2}, B S_{1}$ and $B C_{2}$ ) were evaluated for each cross. Complex gene action was detected with additive gene effects predominant and significant dominant effect in three of the four populations. Jennings et al. (1974b) detected significant general combining ability in a diallel study for resistance to 2ECB.

The difference in gene action may reflect the nature of different populations, but may also be caused by the testing environments. Research on individual loci may provide valuable information on the nature of genes
for resistance to 2 ECB .
The primary objectives of this study were: 1) to use RFLPs to locate QTL for host-plant resistance to 2ECB in maize; 2) to estimate the type of genetic effects for host-plant resistance to 2ECB; and 3) to compare estimates of QTL location and effects across environments.

The secondary objectives were 1) to use RFLPs to locate QTL for three morphological traits, including plant height, ear height, growing degree days (heat units) to anthesis and silk emergence; 2) to estimate the type of genetic effects for these morphological traita; and 3) to compare estimates of QTL locations and effects across environments.

MATERIAL AND METHODS

## Population Development and Experimental Scheme

The population used for RFLP linkage map development and field evaluation was derived from crossing inbred lines Mol7 and B52. Mol7 has been extensively used in commercial hybrid production and breeding programs because of good combining ability for grain yield with inbred lines from Reid Yellow Dent (Hallauer et al., 1988). Mo17 has desirable agronomic traits, but is highly susceptible to feeding damage by 1ECB and 2ECB. Inbred 852 was released in 1959 as a source of resistance to 2ECB (Pesho and Dicke, 1961). B52 has not been used much in seed production or breeding programs due to low combining ability for grain yield and undesirable agronomic traits (Guthrie, 1987). The $F_{2}$ population was obtained by selfing a single $\mathrm{F}_{1}$ plant ( B 52 x Mol7) hybrid. An unselected sample of $150 \mathrm{~F}_{2}$ plants was self-pollinated to produce $\mathrm{F}_{2: 3}$ families. For each $F_{2: 3}$ family, 15 kernels were planted in the greenhouse to obtain leaf tissue for DNA isolation in RFLP analysis. Remnant seed was used for replicated evaluation over environments.

## Experimental Design and Field Layout

A $12 \times 13$ rectangular lattice design included $150 \quad F_{2: 3}$ lines and six checks. The six checks included two plots each of Mo17, B52 and the $F_{1}$. The lattice had two replications in each of the three environments, Ames and Ankeny in 1989, and Ames in 1990. The experiment numbers for the three environments were:

| Experiment | Location/Year | Environment Code |
| :---: | :---: | :---: |
| 89102 | Ames, 1989 | En the Digsertation |
| 89302 | Ankeny, 1989 | Env. 1 |
| 90102 | Ames, 1990 | Env. 2 |
|  | Env. 3 |  |

The lattice design was used to remove environmental variation within the replication because a large number of entries were included in each replication. Plots consisted of two hills with 30 -inch centers at Ames and 40-inch centers at Ankeny. Plots were hand-planted and thinned to three plants per hill. The planting dates were April 25, May 11 and May 29 for Env. 1, 2 and 3, respectively.

## Infestation and Screening for 2ECB

The hill plots were infested during anthesis with newly hatched corn borer larvae (Guthrie, 1987). Twelve applications with approximately 50 larvae per application were applied over seven days for a total application of 600 larvae per plant. The infestation was applied in the leaf axil of the primary ear and the axis of two leaves below and above the primary ear. This rate was higher than that for common breeding and germplasm screening programs to minimize host-plant escapes. Fifty to 60 days after infestation, stalks were split longitudinally from the soil level to the node above the primary ear. The length of larval tunnelling was measured to the nearest inch (ECB). Parallel tunnels were counted once.

The other traits measured were plant height (PT) and ear height (ET) in all environments, and flowering as anthesis and silk emergence dates in Env. 1 and Env. 3. Plant height (cm) and ear height (cm) were measured from soil level to the top of the tassels and to the node of the primary ear after anthesis. Anthesis and silk emergence dates were recorded when $50 \%$ of the plants in each plot reached anthesis and silk emergence,
respectively. Anthesis and silk emergence dates were recorded as days after June 30 (TS and SL for anthesis and silk emergence date, respectively), and translated into Growing Degree Days (GDD, ATS and ASL for anthesis and silk emergence date, respectively; Shaw, 1988) according to the following equation:
$\operatorname{GDD}\left(F^{\circ}\right)=[($ daily maximum temperature+daily minimum temperature)/2]-50. Maximum and minimum temperatures were $86^{\circ} \mathrm{F}$ and $50^{\circ} \mathrm{F}$, respectively. Entry means for traits were recorded in Appendix 5.

## RFIP Analysis

Each $F_{2: 3}$ line was planted in three pots with five seeds per pot in the greenhouse. Leaf tissue samples were collected from eight to 15 seedlings in each $F_{2: 3}$ line to represent the genotype of the progenitor $F_{2}$ plant. An equal quantity of tissue from each seedling was bulked, lyophilized, ground to a fine powder and stored at $-20^{\circ} \mathrm{C}$. Eight hundred grams of leaf powder were used for DNA isolation. Total genomic DNA was isolated from lyophilized tissue following the procedure described by Saghai-Maroof et al. (1984) using CTAB (mixed allytrimethyl-ammonium bromide) extraction buffer. Lyophilized tissue and CTAB buffer mixture was kept in a tube at $65^{\circ} \mathrm{C}$ for one hour and cooled for 5 minutes at room temperature. Chloroform:octanol (24:1, 4.5 ml ) was added to the mixture to form an emulsion by inverting the tube for five minutes. The tubes were centrifuged at 3000 rpm for 10 minutes. The top aqueous phase was removed to a conical tube. The solution was treated with RNase for 30 minutes. DNA was precipitated by adding isopropanol to the solution, inverting the tube and transferring to a solution of $76 \%$ ethanol and 0.2 M sodium acetate for 20 minutes. DNA was dissolved in TE buffer. Chloroform:octanol (24:1) was added to the TE buffer containing DNA and the tube was inverted several times and microcentrifuged for five minutes. The top layer was transferred
to a solution of 7.5 M ammonium acetate and $76 \%$ ethanol. The precipitated DNA was transferred to TE buffer. The concentration of DNA samples was determined by spectrophotometric measurement at 260 and 280 nm as follow: [DNA] $(\mu \mathrm{g} / \mathrm{ml})=\mathrm{A} 260 * 50$

DNA samples of $10 \mu \mathrm{~g}$ from each line were singly digested with EcoRI,HindIII or EcoRV in $300 \mu \mathrm{l}$ and probed with low copy maize genomic and cDNA clones to identify probe-enzyme combinations for detecting polymorphism between the two parents. The digestion was conducted in 300 $\mu l$ of digestion mix containing $10 x$ phosphate restriction enzyme buffer, 0.1 M spermidine and one of the restriction enzymes according to the manufacturer's instruction.

The digested DNA samples were electrophoresed in $0.7 \%$ agarose gel with TE running buffer. Bromophenol blue was used as the dye for tracking DNA migration. DNA fragments in the gel were denatured in a $0.2 \mathrm{~N} \mathrm{NaOH}, 0.6$ NaCl solution for 35 minutes on a shaking platform. The gel was transferred to a solution of 0.5 M tris $\mathrm{HCl}, 1.5 \mathrm{M} \mathrm{HCl}$ for 30 minutes on a shaking platform. Capillary transfer of DNA from the gel to a nylon filter (Magnagraph MSI) with $25 \mathrm{mM} \mathrm{NaPO}_{4}$ ( pH 6.7 ) was performed as described by Southern (1975). The filter was washed for 15 minutes with $2 x$ SSC, air dried and baked for two hours at $85^{\circ} \mathrm{C}$.

Clones included in the preliminary screen for polymorphism were selected to provide uniform coverage of the genome based on linkage maps of maize RFLP loci developed by Brookhaven National Laboratory (BNL) (Coe et al., 1988), University of Missouri, Columbia (UMC) (Coe, et al., 1988), Native Plants Incorporated (NPI), Pioneer Hi-Bred International Inc. (PIO) and Iowa State University (ISU).

The maize genomic and cDNA clones were isolated by digesting plasmid DNA with the appropriate enzyme and electrophoresing the digested DNA on a $0.7 \%$ low-melting point agarose. The gel was stained with $0.5 \mu \mathrm{~g} / \mathrm{ml}$ ethidium
bromide and the insert was removed from the gel with a razor blade. Isolated inserts were radio-labeled to approximately $1-4 \times 10^{9} \mathrm{dpm} / \mu \mathrm{g}$ by random-primer synthesis with ${ }^{32} \mathrm{p}$-deoxy CTP (Feinberg and Vogelstein, 1983).

Hybridization followed the procedures of Helentjaris et al. (1985). Filters were prewashed in 0.1 XSSC and $0.1 \%$ SDS at $65^{\circ}$ for 30 minutes. Filters were prehybridized for four hours or overnight at $65^{\circ} \mathrm{C}$ with prehybridization solutions to block the active sites. The prehybridization solution consisted of $6 x$ SSC , $0.5 \%$ SDS, $5 x$ Denhardts, 25 mM NaPO ${ }_{4}$ and 100 $\mu \mathrm{g} / \mathrm{ml}$ salmon sperm DNA. Labeled probes were boiled for five minutes, added to the hybridization bottles containing the prehybridized filters, and incubated at $65^{\circ} \mathrm{C}$ overnight. The unbound probe was removed by a series of washes. Wash $I$, containing $2 x$ SSC and $0.5 \%$ SDS, mainly removed the unbound probe and eliminated or minimized the background hybridization. Wash II, containing 0.1 SSC and 0.1\% SDS, promoted specificity of the hybridization.

Filters were placed on Whatman 3MM paper after washes, wrapped with saran wrap and exposed to X-ray film at $-80^{\circ} \mathrm{C}$ for six to seven days with intensifying screens (DuPont model). The X-ray films were processed according to the manufacturer's (Kodak) instruction.

One hundred and thirteen maize genomic and CDNA clones were selected for this population according to the results of preliminary survey. Each probe was hybridized with each of the DNA samples from the $150 \mathrm{~F}_{2: 3}$ lines. After the filters' patterns exposed the $X$-ray film in dark, the segregation of the $F_{2: 3}$ lines for each probe can be read from the film. Each film was scored twice independently. The band pattern resembling Mol7 and B52 were recorded as "A" and "B", respectively. Heterozygotes, exhibiting bands for both parental lines, were assigned "H". Some lines produced bands not resembling either parents or did not produce readable bands. These instances were recorded as missing data (2.9\%, 557 of 19097 data points).

## Statistical Analysis

All data were entered twice and verified before conducting statistical analysis.

## Biometrical Analysis for Field Evaluation

Most analyses were based on the assumption that the $F_{2: 3}$ families represented a random sample from a population with a normal distribution. This assumption was tested for all traita with PROC UNIVARIATE (SAS institute, 1988) by using the Shapiro-Wilk test. Two statistics described the fit to a normal distribution, $\underline{W}(0<W<1)$ and Prob<W. A small value of $\underline{W}$ indicates lack of fit, i. e., the sample was not from a population of normal distribution. The probability value Prob<W provided a test of significance. For a significance level of $\alpha=0.05$, if Prob<W $>0.05$ there was not sufficient evidence to reject the hypothesis that the sample was from a normally distributed population. If a Prob<W $<0.05$, deviation from a normal distribution was indicated. In this study, deviation from normal distribution was explained by environmental conditions (see Results section), and there was no patterns of deviation, i.e., the curves of the distribution were not show skewed in certain direction. This evidence indicated deviation from normal distribution was not caused by scales of measurement. Transformation was not performed.

Several population parameters were derived simultaneously in the normality test.

M: mean of the data set.
R: range of the sample by subtracting the lowest value from the highest value in the data set, ( $F_{2: 3}$ line means in this study).

CV: coefficient of variation, the sample standard deviation as a percentage of the sample mean. Each trait was analyzed by using PLABSTAT (Utz, 1987), a statistical
analysis program. The general model for lattice design was

$$
\begin{equation*}
Y=R_{i}+B_{i j}+T_{k}+e_{i j k} \tag{1}
\end{equation*}
$$

where

```
R}=\mathrm{ effect of i'th replication
B}\mp@subsup{\textrm{ij}}{}{\prime}=\mathrm{ effect of }\mp@subsup{j}{}{\mathrm{ th}}\mathrm{ block in ith replication
Tk}=the effect of k k treatment, the line in
    this case
e}\mp@subsup{\mathbf{ijk}}{}{=}\mathrm{ intrablock error
```

The analysis of variance was conducted as described in Appendix 8.
PLABSTAT calculated the ANOVA and the adjusted treatment means. The efficiency of using Lattice Design relative to Random Block Design (RBD) was given as Relative Efficiency (R.E.)
R.E. $=\left(S S_{\text {BLK }}+S S_{\text {intrablock }}\right) /[r(k-1)+(k-1)(r k-k-1)$

If the R.E. was smaller than 105\%, the lattice design did not sufficiently improve the efficiency of the test (Gomez and Gomaz, 1984). Therefore, the efficiency obtained by blocking did not compensate for the losses of the degrees of freedom by blocking, so a RBD was used. In these cases, the final analysis for QTL location were conducted by using unadjusted means. If the R.E. was larger than 105\%, adjusted means were used to remove the interblock effects. ANOVA for combined data over three environments was conducted as suggested by Cochran and Cox (1957).

The lattice analysis of variance for individual environment data indicated that the significance of block effects varied among traits and environments. The efficiency of blocking and significance of the block effect for each trait in each environment were as follows:

Traits

|  | PT | ET | $C B$ | TS | SL | ATS | ASL |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | - R.E. - |  |  |  |  |  |
| Env. 1 | 100 | 107 ${ }^{\text {m }}$ | 105* | 101 | 101 | 102 | 101 |
| Env. 2 | 100 | 100 | 102 |  |  |  |  |
| Env. 3 | 204 ${ }^{-}$ | 122m | 119" | $128^{\prime \prime}$ | 113** | 121 ${ }^{-}$ | $106^{-7}$ |

*, **: block effect significant at 0.05 and 0.01 level, respectively. According to these results, the final analysis and mapping were conducted with the adjusted means from lattice design for ET and 2ECB in Env.1, and all traits in Env. 3. Unadjusted means were used for others.

The ANOVA for RBD was summarized in Appendix 8. Sufficient variation among the $F_{2: 3}$ lines in ANOVA was prerequisite for conducting further analysis. The heritability on the basis of $F_{2: 3}$ line means was calculated as suggested by Hanson (1963). For estimation in one environment:

$$
\begin{equation*}
h_{F 3}^{2}=\frac{\hat{\boldsymbol{\sigma}}_{g}^{2}}{\hat{\sigma}_{g}^{2}+\hat{\sigma}_{e}^{2} / r} \tag{3}
\end{equation*}
$$

where

$$
\begin{aligned}
& \hat{\sigma}_{\mathrm{s}}^{2}=\text { genetic variation component } \\
& \hat{\sigma}_{\mathrm{a}}^{2}=\text { error variation } \\
& r=\text { number of replications in each environment }
\end{aligned}
$$

The same terms were used throughout the dissertation (Appendix 8). For estimation across environments:

$$
\begin{equation*}
h^{2}=\frac{\hat{\sigma}_{g}^{2}}{\hat{\sigma}_{g}^{2}+\hat{\sigma}_{g \theta}^{2} / e+\hat{\sigma}_{\theta}^{2} / r e} \tag{4}
\end{equation*}
$$

where $\dot{\sigma}_{\mathrm{p}}^{2}{ }^{2}=$ component of genetic x environment variation
e = number of environments
Confidence intervals for the estimates of heritability were calculated according to Knapp et al. (1985):
$P\left\{1-\left[(M 1 / M 2) F_{1-\alpha / 2: d 2, d 7}\right]^{-1}<=1-\theta_{2} / \Theta_{1}<=1-\left[(M 1 / M 2) F_{\alpha / 2: d 2, d n}\right]^{-1}\right\}=1-\alpha$
where $M 1$ and M2 correspond to mean squares terms in ANOVA in Appendix 8. As Knapp's derivation was based on the availability of two mean square terms to derive estimate of heritability, M1 and M2 represented the two mean square terms for all cases. M1 and M2 for ANOVA in single environment and across environments were specified in Appendix 8.
$\alpha$ : significance level
df1 and df2: degree of freedom of $M 1$ and $M 2$, respectively
$\theta_{1}$ and $\Theta_{2}$ : true values of $M 1$ and $M 2$.
Estimates of correlation among traits were derived as follows
(Falconer, 1989):
Phenotypic Correlation

$$
\begin{equation*}
r_{p_{x_{1} x j}}=\frac{\hat{\sigma}_{x_{1} x_{j}}}{\hat{\sigma}_{x_{1}} \hat{\sigma}_{x_{j}}} \tag{5}
\end{equation*}
$$

Let $\quad \mathrm{Xi}_{\mathrm{i}}=$ phenotypic value of ith trait
then $\quad \hat{\sigma}_{x i x j}=$ covariance for $X_{i}$ and $X_{j}$.

$$
\hat{\sigma}_{x i}=\left(\sigma_{x i}^{2}\right)^{1 / 2}=\text { square root of phenotypic variance of } X_{i} \text {. }
$$

Genetic Correlation

$$
\begin{equation*}
r_{g_{i}, g_{j}}=\frac{\hat{\sigma}_{g_{i} g_{j}}}{\hat{\sigma}_{g_{1}} \hat{\sigma}_{g_{j}}} \tag{6}
\end{equation*}
$$

Let $\quad G i=$ genotypic value of ith trait
then $\hat{\sigma}_{\operatorname{gikj}_{j}}=$ covariance for $G_{i}$ and $G_{j}$.
$\hat{\sigma}_{\dot{\alpha}}=\left(\hat{\sigma}_{g_{i}}\right)^{1 / 2}=$ the square root of phenotypic variance of trait $G_{i}$. The analysis was done in CORR and GLM Procedures in SAS (SAS Institute,
1988).

Statistical Analysis for Marker Data
Segregation ration of individual markers were tested among $150 \quad \mathrm{~F}_{2: 3}$ lines because Mendelian segregation was assumed for the mapping procedures. The codominant nature of RFLP markers gave three distinguishable genotypic classes at each locus among $F_{2: 3}$ lines, two homozygous classes and $a$ heterozygous class with a ratio of $1: 2: 1$, as expected for an $F_{2}$ population produced from two inbred and highly homogeneous parents. The 1:2:1 ratio for $A, H$ and $B$ was used as the hypothesis tested in a chi-square statistic calculated as

$$
\begin{align*}
& \chi^{2}=\Sigma \frac{\left(O_{i}-E_{i}\right)^{2}}{E_{i}}  \tag{7}\\
& i=1,2, \ldots \ldots n \text {. } \\
& \text { where } \quad n=\text { number of } F_{2: 3} \text { lines included in the test } \\
& 0=\text { observed number of individuals in each } \\
& \text { marker class } \\
& \mathrm{B}=\text { expected number of individuals in each } \\
& \text { marker class }
\end{align*}
$$

A SAS program for chi-square test written by K. Lamkey (1991, unpublished) was modified on Unix SAS and used for the analysis. The test results for all probes were listed in Appendix 4.

The principle of constructing the linkage map was the same as classical two-point and three-point mapping (Fristorm and Clegg, 1989). The two parental and heterozygous band patterns on autoradiograms provided the segregating phenotypes used to establish the map. A pair of loci that did not fit a segregation ratio of 9:3:3:1 indicated the possibility of linkage between the two loci; in other words, they were not independently inherited. The distance between the two loci was expressed as the crossover value between two markers which was indicated by the ratio of
recombinant classes in the progeny. If three loci were studied simultaneously, the order of the markers in the linkage group was deduced from the distance of each pair of markers. In this case, double crossovers could be detected, which gave more precise estimates of the genetic distances.

The genomic composition of each $F_{2}$ plant was estimated following the method described by Paterson et al. (1988, 1991). The components for genomic composition were three genotypic classes at a locus (A, $B$ and $H$ ) in the $F_{2}$ population and recombination distances between loci. When consecutive markers along the chromosome of an individual had the same genotype, it was assumed that region between two markers was comprised entirely of that genotype. When consecutive markers revealed different genotypes, the interval was assumed to be comprised of equal contributions of each genotype. The composition of each $F_{2: 3}$ line was calculated. The range and average of the genomic composition of $F_{2: 3}$ lines were obtained.

Data from the probes were analyzed to determine the linkage group by two-point procedure and the order of the probes in each linkage group was determined by three-point analysis. This process was facilitated by MAPMAKER program (Version 2.0) (Lander et al. 1987; Lincoln et al., 1990). The parameters used to differentiate linkage groups by two-point test were LOD threshold 3.5 and recombination value of 0.3 . One hundred and six probes were assigned to 10 linkage groups. Three-point analysis was conducted to determine the order of probes in each linkage group using a LOD of 3.0 as the exclusion threshold. Seven unlinked probes were assigned to the 10 groups by relaxing the recombination value gradually until all probes were included (Vallejos et al., 1992). The largest interval reaches 80 cM on chromosome 10. Haldane function (Haldane, 1919) was used to convert recombination to centimorgan as Lander and Botstein (1989) described.

Putative QTL for all traits were detected and located using two procedures, linear regression and interval mapping. Two procedures were used to provide complementary information. Interval mapping provided an estimate of the relative location of QTL in the interval flanked by two marker loci. Single marker analysis only indicated an association of marker loci with QTL. On the other hand, single marker analysis provided a test of significance for total marker effects and additive and dominance effects. This information may be desired by breeders who were interested in the variation components of the effects. Interval mapping identified regions containing putative $Q T L$, and the potential contribution of the regions to the trait expression was indicated by LOD scores and coefficient of determination $R^{2}$. In a $F_{2}$ population derived from two inbred lines $A$ and B, the phenotypic value of $i^{\text {th }}$ individual can be given by :

$$
\begin{equation*}
Y_{i}=\mu+n_{i} a+h_{i} d+e \tag{8}
\end{equation*}
$$

where
$\mu=$ the mean value of the component of the trait not controlled by this QTL, the average value of AA individuals
$a=$ the additive component of the QTL B allele effect
$n_{1}=$ the number of $B$ alleles carried by the $i^{\text {th }}$ individual $(0,1$, or 2)
$d=$ the dominance component of the QTL B allele effect
$h_{1}=$ taking value of 1 when $i^{\text {th }}$ individual is $A / B$, and taking value of 0 otherwise
$e=$ error of a normal random variable The field evaluation was conducted among $F_{2: 3}$ lines, while the inference was made to a $F_{2}$ population. Therefore, the eatimates of dominance effects were adjusted by multiplying the constant of 2 (Mather and Jinks, 1982).

Multiple loci analysis in interval mapping examined multiple QTL simultaneously, extending the sensitivity of QTL mapping (Lincoln et al.,

1990b). The analysis assumed independent qTL act additively explained by the following model for two loci:

$$
\begin{equation*}
Y_{1}=\mu+n_{1} a_{1}+h_{i} d_{1}+n_{1} a_{2}+h_{1} d_{2}+e \tag{9}
\end{equation*}
$$

where subscripts 1 and 2 indicating locus 1 and 2, respectively. Estimates of the QTL effects were slightly more accurate than the estimates in the analysis for individual loci because the amount of unexplained error by the model was reduced. Multiple loci analysis indicated the relative position of gTL to the two markers defining the interval. Estimates of gene effects showed the direction of gene action. Single marker analysis can be used to confirm the results from interval mapping, and provide a test for statistical significance for additive and dominant genetic variation. Operation of the procedures was according to the following description. Interval mapping was described by Lander and Botstein (1989). The traditional method was a special case of the maximum likelihood used to define the probability for interval mapping.

Let $\phi_{\mathrm{l}}=$ Phenotype for $\mathrm{i}_{\mathrm{th}}$ individual $g_{i}=$ genotype for the $i_{\text {th }}$ individual

Assume $\phi_{i}$ and $g_{i}$ were related by the equation

$$
\begin{align*}
& \phi_{i}=a+b g_{i}+e  \tag{11}\\
& e^{-N}\left(0, \sigma^{2}\right) .
\end{align*}
$$

where $a$ can be interpreted as the population mean and $b$ the regression coefficient of the model.

Regression had the same expression as Fisher's illustration for resemblance between relatives and $b$ was the estimated phenotypic effect of a single allele substitution. The linear regression solution ( $a, b, \sigma^{2}$ ) was the maximum likelihood estimate (Mr.E) maximizing the probability $L\left(a, b, \sigma^{2}\right)$ that the observed data would occur.

$$
\begin{equation*}
L\left(a, b, \sigma^{2}\right)=\pi_{i} z\left(x, \sigma^{2}\right) \tag{12}
\end{equation*}
$$

where

$$
x=\phi_{i}-\left(a+b g_{i}\right)
$$

$$
z\left(x, \sigma^{2}\right)=\left(2 \pi \sigma^{2}\right)^{-1 / 2} * \exp \left(-x^{2} / 2 \sigma^{2}\right)
$$

Assume $\mathrm{b}=0$, the constrained MLEs were $\left(\hat{\mu_{a}}, 0, \hat{\sigma}_{\mathrm{Bi}}{ }^{2}\right.$ ) which indicated no QTL was linked. Assume $b=1$, the constrained MLEs were ( $a, b, \sigma^{2}$ ) $(b \neq 0)$ which implied the linkage between a marker and QTL. The evidence for putative QTL was summarized in LOD score which was defined as

$$
\begin{equation*}
\operatorname{LOD}=\operatorname{LOG}_{10}\left[\hat{L}\left(\hat{a}, \hat{b}, \sigma^{2}\right) / \hat{L}\left(\mu_{A}, \hat{0}, \sigma_{B}^{2}\right)\right] \tag{13}
\end{equation*}
$$

The LOD score essentially indicated how much more probable the data were to have arisen assuming the presence of a QTL as opposed to assuming the absence of QTL. The procedure assumes Mendelian segregation and a random sample from a normally distributed population. The logarithmic function was used in the MLE procedure to simplify the calculation. The choice of $\log _{10}$ was used because this method was invented for human genetics where the common logarithm was a practice subsequently adapted to investigations in plant breeding (Paterson et al., 1990; Tanksley et al , 1989) .

Since genetic markers have not been identified throughout the genome, RFLP markers for arbitrary locations in the genome were not available to detect QTL using the method defined at arbitrary points. Interval mapping provided an alternative by scanning the whole genome at intervals defined by pairs of markers.

The likelihood function used for interval mapping was defined as

$$
\begin{equation*}
L\left(a, b, \sigma^{2}\right)=\pi_{i}\left[G_{i}(0) L_{i}(0)+G_{i}(1) L_{i}(1)\right] \tag{14}
\end{equation*}
$$

where $\quad L_{l}(x)=z\left[\left(\phi_{i}-(a+b x), \sigma^{2}\right]\right.$ denoted the likelihood function for individual $i$ assuming that $g_{i}=x$, and $G x$ was the probability of $g_{i}=x$ conditional on the genotype and positions of flanking markers.

Linear regression with known probability distribution but unknown values of genotypes could be used to obtain the MLE solution ( $a^{*}, b^{*}, \sigma^{2 n}$ ). However, this solution could only be derived through maximization methods
instead of least square estimation. MAPMAKER/QTL has employed techniques for maximum likelihood estimation with missing data. This method was proper because the genotype could be treated as missing data in the maximization procedure (Lander and Botstein., 1989; Lincoln et al., 1990b).

In statistical procedures, the significance level $\alpha=0.05$ was commonly used because a reasonable balance of type I and II error could be achieved at this level (Snedecor and Cox, 1989). As a large number of tests were conducted simultaneously when the entire genome was tested, a significance level of 0.05 , corresponding to a LOD score of 0.83 , would lead to a high chance of Type I error (Lander and Botstein, 1989). The consequence would be identification of too many significant $Q T L$; in other words, false positives would be reported.

Lander and Botstein (1989) described the significance level used in interval mapping as a threshold for detecting putative gTL and illustrated the derivation of an appropriate threshold that depended on the size (CM) of the genome and the average density of markers over the genome. Two cases should be considered. 1)First in the case of the sparse-map, the consecutive markers were well separated, thus, the occurrences of spuriously high LOD scores were independent statistical events. If the significance level was $\alpha$ with $m$ intervals, standardized random variable for individuals had a value of $z$, a nominal significance level of $\alpha / \mathrm{m}$ for each individual test corresponds to a LOD threshold of

$$
1 / 2(\text { lge })\left(z_{a / m}\right)
$$

2) In the case of dense-map, the occurrence of spuriously high LOD score at nearby markers were not independent events.

Let $C=$ number of chromosomes of the organism
$G=$ total genetic length in $C M$
$t_{\alpha}=$ solutions for the equation

$$
\alpha=\left(C+2 G t_{\alpha}\right) x^{2}\left(t_{\alpha}\right)
$$

where $x^{2}(t)=F(t)$ for the inverse Chi-square distribution. The appropriate LOD threshold is expressed as $T_{\alpha}=(2 \ln 10) t_{\alpha}$.

As investigation of independence among markers was a mathematically complicated event, Lander and Botstein (1989) provided results from a large scale simulation study for selecting proper LOD scores for species with different number of chromosomes and average distances between each pair of markers. For maize with 10 chromosomes, the threshold would fall between 2.5 to 3 for density of 10 to 15 cm with the significance level of 0.05 according to the simulation by Lander and Botatein (1989). The result can be obtained using the derivation for dense map. The larger the interval becomes, the smaller threshold should be used. Based on the linkage map developed from the population in this experiment, the threshold LOD score for QTL location was set at 2.5 .

The analysis was conducted with MAPMAKER/QTL 2.0 (Lincoln et al., 1990b). The free genetics model (described by equation 8) was used to scan over the whole genome without constrains. Unconstrained model was used because of the following reasons: 1) The free model generated the optimum results. The constrained models can only be used to rule out other possibilities; 2) Multiple loci analysis can only be applied to free model. If more than one region was suggested on one chromosome within 50 cm , each region was analyzed with other regions fixed for individual contribution. Multiple loci analysis (Lincoln et al., 1990b) including all regions detected in genome scan was conducted to obtain the results when all regions acted simultaneously. The relative position of putative QTL to the two markers defining the interval was decided in the analysis. Because the additive model was assumed for multiple loci analysis (equation 9), difference between LOD score from multiple loci analysis and the sum of individual LOD scores in genome scan might suggest epistasis among regions;
however, this difference might also caused by failing to meet other assumptions for the analysis.

Single marker analysis was conducted according to the following procedure with a linear regression model. Phenotype of traits (Y) were the trait values based on $F_{2: 3}$ line means. For a locus with two alleles, a linear model for the phenotype was proposed by Mather and Jinks (1982) and first used with molecular marker in maize by Edwards et al. (1987) and Stuber and Edwards (1987) as follows:
$\mathbf{Y}=\mathbf{a}+\mathbf{d}+\mathbf{e}$
where $\quad \underline{a}=$ additive effect
$\underline{d}=$ dominant effect
e $=$ random error of the model
Three marker classes at each locus gave one degree of freedom to and $\underline{a}$, respectively.

Regression of $F_{2: 3}$ phenotype on the marker genotype provided the test to determine if different marker classes were associated with variation of phenotypes. The association of molecular marker classes and variation of phenotypes suggested linkage between the marker and QTL for the traits of
interest. The regression model was

$$
\begin{equation*}
\mathbf{Y}=M+e \tag{16}
\end{equation*}
$$

where $\quad Y=$ phenotype of the trait, i.e. trait value
$M=$ effect of marker class
$e=$ random error of the model
Analysis of variance for the regression (Steel and Torrie, 1960) had the form in Appendix 9.

A significant regression mean square $\left(M S_{R}\right)$ indicated the marker being tested was linked to the gene(s) conditioning trait $Y$. A significance level of $\alpha=0.001$ was used. Since 113 tests were conducted, the exact overall significance level of 0.1 for individual tests was 0.0009 on a per
contrast basis. This was rounded to 0.001 as a proper significance level for individual tests.

The total regression variation can be partitioned into two components, additive and dominant genetic variation. These components can be derived by two orthogonal comparisons (Cochran and Cox, 1957).

Additive component: Contrast $1 \quad C_{1}=A-B$
Dominant component: Contrast $2 \quad \mathrm{C}_{2}=\mathrm{H}-1 / 2(\mathrm{~A}+\mathrm{B})$
where $A=$ phenotypic class for homozygous for parent $A$
B=phenotypic class for homozygous for parent B
H=phenotypic class for heterozygous for parents $A$ and $B$
This partition of total variation was the same as the regression explained by Fisher: additive variation was the variance of regression of the phenotype on the number of effective alleles and dominance variation was the residual from the regression (Falconer, 1989). Statistical analysis for equation (16) and two comparisons in equation (17) and (18) were conducted in General Linear Model Procedure on Unix SAS (SAS Institute, 1988). Significance of genetic variation derived Equation (16) indicated by single marker analysis was defined by the following terms: A indicated significant additive variation, i.e. variation from Equation (17) was significant when tested against regression residual ( $\alpha=0.001$ ); $D$ indicated significant dominance variation, i.e. variation from equation (18) was significant when tested against regression residual ( $\alpha=0.001$ ); A/D indicated significant additive and dominance variation with additive variation predominant, i. e. $p_{A}<p_{D} ;$ and $D / A$ indicated significant additive and dominance variation with more dominance variation, i.e. $p_{D}<p_{A}$. These were listed in the results section as gene action (see Results section Table 4).
Construction of Linkage Map for Population Mol7 X 852 Test for segregation
The chi-square test (Appendix 4) indicated that among 113 probes used, 89 fit the 1:2:1 segregation ratio at $\alpha=0.05$ level. Ratios of genotypic classes at 21 loci( 18.6\%) deviated from the expectations (Table 1). Those loci were located on 7 chromosomes (Figure 2). Only four (3\%) reached $\alpha=0.01$ level . The most extreme case was chromosome 7 with seven linked probes exhibiting deviation from the expected segregation ratio. Deviation was attributable to excess or lack of certain genotype(s), as the gene frequency of parental alleles was between 0.4 to 0.6 for all loci. Deviations from expected segregation ratios have been reported in other molecular marker-based mapping studies (Edwards et al., 1987; Paterson et al., 1990).

## Genome Composition

Overall, the $F_{2: 3}$ lines were $24.1 \%$ homozygous for Mol7 genome, 23.2\% homozygous for $B 52$ genome and 52.7\% heterozygous on average (Figure 1). These values closely matched the theoretical composition of $25 \%, 25 \%$ and $50 \%$ for Mo17, B52 and B52 x Mol7, respectively. Percentage of Mol7 genome and $B 52$ genome ranged from $0 \%$ to $50 \%$ and $0 \%$ to 45\%, respectively. Percent of heterozygosity ranged from 28\% to $84 \%$. The distribution of the genomic percentage was symmetric about the means for all three classes in accordance with expectation for normal random variables. All probes were used to construct the linkage map.

## Genetic Linkage Map

The linkage map was constructed with 113 probes and comprised 1504.5 $C M$ (Figure 2). The average distance between each pair of loci was 13.3 cm . Regions with intervals larger than 35 cM were constructed by relaxing the
recombination value (r). Relaxation of $r$ allowed an increased spacing between markers in the same linkage group and consequently several gaps appeared in the map, BNL5.62-BNL12.06 (47.3 cM) and UMC157-UMC67 (35.7 cM) on chromosome 1, UMC121-UMC50 (56.2 cM) and NPI457-NPI250 (56.6 cM) on chromosome 3, BNL5.27-NPI292 (60.2 cM) on chromosome 4, BNL14.28-BNL8.17 (53.0 cM) and BNL3.06-CI (38.3 cM) on chromosome 9 and UMC64-PIO20.0075 ( 80.9 cM ) on chromosome 10.

## Biometrical Analysis for 2ECB Tunnelling

$F_{2: 3}$ lines fit a normal distribution for 2ECB tunneling in two of the three environments (Table 2 and Appendix 10). In 1990, possibly due to the infestation of $a$ fungus in the maize gtalks, Beauveria bassiana, the tunnelling count was relatively low for Environment 3 . The range of $F_{2: 3}$ line means for tunnelling was 13 inches for Environment 3 and 26 and 23 inches for Environments 1 and 2, respectively. Parental lines Moll and B52, and the singlemcross hybrid averaged $24.6,12.6$, and 14.3 inches for 2ECB tunnelling over all environments, respectively. The degree of 2ECB tunnelling exhibited by the $F_{1}$ indicated dominant gene action for some of the QTL conferring host-plant resistance.

Genetic variation for ECB tunnelling was significant in each environment. Heritability estimates were $62.2 \%$ and 68.5\% for Environments 1 and 2 (Table 3). The heritability estimate of $47 \%$ in Environment 3 was relatively low due to the lack of differentiation among $F_{2: 3}$ lines for ECB tunnelling. The low level of 2 ECB damage was reflected through the tunneling length of susceptible parent Mol7 (Appendix 5). Significant genotype by environment interaction was detected.

QTL Location for 2ECB Tunnelling
LOD scores for inches of 2ECB tunnelling were plotted for each linkage group in each environment (Figure 3). A unique set of putative QTL were identified in each environment (Table 4). Alleles from B52 contributed to reduced 2ECB tunnelling and accounted for a majority of the phenotypic variation; however, Moll seemed to contribute resistance in several instances.

In environment 1 four regions representing four chromosomes had a LOD score exceeding 2.5. Three of the four regions, and those with the largest effects were derived from B52. The region on chromosome 1 had the highest LOD of 5.2. Additive and dominance effects were estimated as -2.3 inches and -2.0 inches, respectively. The total phenotypic variation in the population explained by this region was 18\%. Substitution of two doses of alleles on this region from B52 into Mol7 would reduce the corn borer damage approximately 4.75 inches. The range of 26.4 (40.1-13.7) inches in environment 1 reflected the total phenotypic variation in the population. Of this total difference, $18 \%$ could be explained by the region on chromosome 1. Assuming the highest damage was in Mol7 and lowest damage was in B 52 , substitution of this region would change the mean by 4.75 (26.4×18\%) inches. Multiple loci analygis indicated QTL for 2ECB were closer to BNL15.18 (2.9 cM from BNL15.18, 27 CM from BNL8.29). The negative additive effect ( $\mathbf{- 2 . 3 )}$ indicated that alleles for resistance were derived from B52. Dominance effect of $\mathbf{- 2 . 0}$ indicated the genotype of heterozygote had less corn borer damage than the homozygous Mol7 genotype. Single marker analysis confirmed QTL position near BNL15.18 (probability 0.0001 for BNL15.18, 0.0236 for BNL8.29), and indicated additive and dominance variation contributed to genetic variation. On chromosome 2, single marker and multiple loci analysis indicated QTL for 2ECB between UMC135 and UMC131, 10 cM from UMC135 with mainly dominance gene effects.

The variation explained by this region was estimated at 14\%. Additive and dominance effects were estimated as -1.8 and -1.6 inches, respectively. The region identified on chromosome 9 had a LOD of 3.7 and estimates of additive and dominance effects of -1.9 and -2.6 inches, respectively. Single marker analysis identified four very closely linked markers, UMC114, UMC20, UMC81, and UMC153 with predominantly additive variation. Multiple loci analysis indicated UMC114 might be the most precise position for the QTL. The phenotypic variation explained by this region was $10 \%$. The fourth region with QTL for 2ECB was on chromosome 10. Multiple loci analysis suggested QTL were between NPI287 and NPI232. The only marker detected by single marker analysis was NPI232. Contrary to the three other regions, QTL for resistance of this region were derived from Mo17. Additive and dominace effects were estimated as 1.3 and $-3,2$ inches, respectively.

The LOD score from the multiple loci procedure (13.3) was less than the sum of LOD values from individual intervals (16.5). The difference between these two values suggested possible interaction among loci.

Seven regions were detected with LOD larger than 2.5 in Environment 2. Chromosome 1 had a region with a LOD of 5.0 accounting for $15 \%$ of the phenotypic variation. Additive and dominance effects were estimated as 1.8 and -1.8 inches, respectively. Substitution of Mo17 alleles with 852 alleles in this region would cause reduction of corn borer damage by 1.8 inches if only additive effects for this region were considered. Single marker and multiple loci analyses indicated the most likely position of QTL was near UMC128 within 8.6 cM of the next locus, UMC23. Significant additive and dominant components of variation were detected. Dominance appeared to be more important. On chromosome 2 , the region containing QTL for 2ECB was marked by AGP2 and NPI585 2.9 cM . This region exhibited mainly dominant variation in single marker analyais. The total variation
explained was 8\%. Estimates of additive and dominance effects were 1.9 and 1.4 inches. Mo17 contributed resistance for this region. The region on chromosome 3 was marked by UMC26 and BNL5.37. QTL were closer to BNL5.37 according to multiple loci and single marker analyses. Additive variation contributed more to total genetic variation in this region. Total variation explained was 8.6\%. Estimates of additive and dominance effects were -1.6 and -3.6 inches. Alleles for low tunnelling length in this region were derived from B52. The region on chromosome 4 (LOD=3.2) was marked by BNL15.07 and NPI203 and accounted for $11.4 \%$ of the phenotypic variation. Estimates of additive and dominance effects were 1.3 and 1.6 inches, respectively. Additive and dominant components of variation were of equal importance. Three markers on chromosome 8 were identified in the region with a LOD of 5.4. Multiple loci and single marker analyses indicated BNL9.08 and BNL9. 44 represented this region with BNL9.08 closest to the QTL. additive and dominance effects were estimated as $\mathbf{- 2 . 2}$ and $\mathbf{- 1 . 0}$ inches, respectively. Additive variation seemed to be predominant. The phenotypic variation attributable to this region was 15.3\%. QTL on chromosome 9 were indicated by a cluster of closely linked markers, UMC114, UMC20, UMC81 and UMC153. Estimates of additive and dominance effects were -2.1 and -1.0 , respectively. Additive and dominant components of variation were important. The phenotypic variation explained by this region was 11.2\%. On chromosome 10, the putative QTL were flanked by UMC64 and PIO20.0075. This region did not have an adequate density of markers. In this case, single marker analysis might provide more valid information. In the region detected by interval mapping, single marker analysis detected PIO20.0075 with significant additive variation ( $p=0.0005$ ) . This result indicated QTL must be close to PIO20.0075, restricting QTL to a narrower region. Resistance was derived from Mol7. Overall, the sum of LOD scores from individual loci (25.7) was similar to the LOD from multiple loci
mapping (25.1) indicating an additive relationship among loci.
In Environment 3 three chromosome regions, all derived from B52, were detected with LOD larger than 2.5. Chromosome 2 had a region with a LOD of 2.8. Additive and dominance effects were estimated as -1.1 and 0.4 inches, respectively. Multiple loci and single marker analyses indicated the QTL were closer to UMC78. Dominance variation was more important than additive variation. The total phenotypic variation explained was 12.3\%. The region identified on chromosome 7 had a LOD of 3.9. UMC110 and BNL15.21 defined the QTL with predominant additive variation in an interval of 16 cM . The phenotypic variation explained was 13.1\%. Estimates of additive and dominance effects were -1.2 and -1.6 , respectively. Chromosome 9 had an interval with predominant additive variation. This region could be represented by UMC20, UMC81, UMC153 and BNL3.06. The phenotypic variation contributed by this region was 9.0\%. Estimates of additive and dominance effects were -0.9 and -1.6 , respectively. The large discrepancy between LOD score for multiple loci analysis (18.4) and the sum of LOD scores for individual intervals (9.8) might suggest epistasis among loci and the increased LOD in the multiple loci analysis implied a positive interaction.

## Biometrical Analysis for Morphological Traits

$F_{2: 3}$ line means fit a normal distribution for all traits in Environment 1 except for ATS (GDD), and for all traits in Environment 2. $\mathrm{F}_{2: 3}$ line means in Environment 3 for PT, ET, ATS and ASL deviated from normal distribution (Table 5 and Appendix 10). Environment 3 was exposed to extreme climatological changes during the season. Some plots were under water early in the season and development of plants was delayed. Plots experienced drought later in the season. These factors might contribute to the deviation from normal distribution.

Genetic variation was highly significant for all traits. Genotype by
environment interaction was highly significant for PT and ET, significant for ATS, not gignificant for ASL (Table 6). Heritability egtimates were between $54 \%$ to $85 \%$. Most of the estimates were larger than 60\%.

Phenotypic correlations were calculated for individual environments and combined data (Table 7 and 8). Correlations between PT and ET were high ( $r>0.7$ ) in all three environments. Correlations between ATS and ASL were high in Environment 1 and in the upper boundary of medium ( $r=0.68$, 0.66 ) in Environment 3. 2ECB had low correlation ( $r<0.2$ ) in most cases.

## Location of QTL for Morphological Traits

QTL for PT on chromosomes 1 and 8 were identified in all environments (Figure 4 and Table 8). These regions had the highest LOD scores and explained the largest amount of phenotypic variation for the trait in individual environments. The region on chromosome 1 was defined by BNL15.18-UMC128 and had peak LOD scores between 5.2 and 8.9. Estimates of additive effects were $-13.6,-11.3$ and -14.9 cm for Environments 1,2 and 3, respectively. Dominance effects were estimated as $1.8,2.4$ and 7.2 cm in Environments 1, 2 and 3, respectively. Substitution of B52 alleles with Mo17 alleles for the region on chromosome 1 would increase plant height 13.6 cm in Environment $1,11.3 \mathrm{~cm}$ in Environment 2 and 14.9 cm in Environment 3. The phenotypic variation explained was 27.3\%, 18.5\% and $32.2 \%$ for Environment 1, 2 and 3, respectively. B52 contributed alleles for shorter plant stature. Additive and dominance components of variation were significant, but dominance variation was more important. The region on chromosome 8, defined by NPI268-UMC89, had LOD scores 4.7, 5.0 and 5.6. The phenotypic variation explained was $16.3 \%, 23.1 \%$ and $19.2 \%$ for Environments 1, 2 and 3, respectively. Additive variation was the most important source of variation. Mol7 contributed the alleles for shorter plant stature. Two regions detected in one environment were UMC21-P11 on
chromosome 6 in Environment 1 and UMC50-UMC121 on chromosome 3 in environment 2. These two regions had relatively small LOD scores (LOD=2.9 and 2.5 for regions on chromosome 6 and 3, respectively). Single marker analysis did not detect QTL on chromosome 3. Additive and dominance effects were estimated $a s-10.6$ and 17.4 cm , respectively. The region on chromosome 6 was indicated by one marker, NPI560 which was identified as the marker closer to QTL in the region by interval mapping. Dominance variation was more important. Estimates od additive and dominance effects were -10.6 and 17.4 cm , respectively. B52 contributed alleles for shorter plants. Single marker analysis identified the same regions as interval mapping for PT in most of the regions (Appendix 10). When the QTL were located in the middle of the interval, single marker analysis tended to have low probability for the two marker loci, such as BNL15.18-UNC28 and NPI268-UMC89 in Environments 1, 2 and 3.

QTL for ET were identified on chromosomes 1 and 8 in three environments on the same intervals as for PT (Table 8 and Figure 5). QTL on chromosome 1 were close to BNL15.18. Estimates of additive effects were $-6.4,-5.9$ and -10.3 cm for Environments 1,2 and 3, respectively. Estimates for dominance effects were $-0.6,-2.6$ and -8.0 cm for Environments 1, 2 and 3, respectively. Substitution of 852 alleles with Mo17 allele on the region on chromosome 1 would cause increase of ET by 6.4 cm in Environment $1,5.9 \mathrm{~cm}$ in Environment 2 and 10.3 cm in Environment 3 if only additive effects were considered. Additive and dominance components of variation were significant. 852 contributed alleles for lower ear height. The region on chromosome 8 was defined by NPI268-UMC89, and mainly contributed additive variation. Alleles for lower ear height were derived from MO17. Two regions were only detected in Environment 1 on chromosomes 6 and 7 , and one region on chromosome 1 was only detected in Environment 2. The region on chromosome 6 had estimates of additive and
dominance effects of -7.0 and -0.02 cm , respectively; the region on chromosome 7 had 7.8 and 8.6 cm . Estimates for additive and dominance effects were -2.5 and -10.6 for the region on chromosome 1 identified in Environment 2 only. Additive variation was more important for all three regions and 852 contributed alleles for lower ear height. Single marker analysis identified the same regions as interval mapping. When the intervals were not very large ( $<35 \mathrm{cM}$ ) and the QTL were identified in the middle of the interval by interval mapping, single marker analysis identified two flanking markers, such as BNL15.18-UMC128 on chromosome 1 and NPI268-UMC89 on chromosome 8. However, when the interval was large ( $\mathbf{> 3 5} \mathrm{cM}$, BNL12.06-BNL5.62 on chromosome 6 ) or the QTL were identified close to one of the two flanking marker loci (PIO10.0016-NPI560), usually one of the two marker loci was detected in the single marker analysis. NPI560 was identified for the interval of PIO10.0016-NPI560 (15.9 cM, QTL were identified 1.9 cM from NPI560). BNL5.62 was identified for BNL12.06BNL5. 62 (47.3 cM, QTL were identified to be 12.3 from BNL5.62).

QTL for ATS (GDD) were identified on a single interval on chromosome 8 in both environments (Figure 6 and Table 10). The region had LOD scores of 8.2 and 8.1 for environments 1 and 2, respectively. Phenotypic variation explained by this region exceeded $20 \%$ in both environments. Additive effects were estimated as 21.4 and 26.2 GDD in Environments 1 and 3, respectively. Estimates for dominance effects were $\mathbf{- 4 . 2}$ and 3.4 for Environments 1 and 3, respectively. Substitution of $\mathbf{B 5 2}$ alleles with Mol7 alleles on this region would prompt early anthesis by 21.4 GDD in Environment 1 and 26.2 GDD in Environment 3 Additive and dominance variation were significant with dominance being more important. Alleles for early anthesis were derived from Mol7. Single marker analysis identified the same regions.

QTL for ASL were defined by four intervals on four chromosomes (Figure

7 and Table 9). Intervals on chromosome 1 and 8 were identified in both environments and the intervals were flanked by the same RFLP markers as for ATS. On chromosome 1, dominance variation was significant. Estimates of additive and effects were 24.0 and 35.8 GDD for Environments 1 and 3, and estimates for dominance effects were -19.6 and -32.0 GDD in Environments 1 and 3, respectively. QTL on chromosome 8 was defined by NPI268-UMC89. Estimates of additive effects were 29.9 and 52.7 GDD for Environments 1 and 3, respectively. Dominace effects were estimated as 10.4 and -43.0 GDD in Environments 1 and 3, respectively. Substitution of B 52 alleles on this region with Mo17 alleles would cause earlier silk emergence by 24 GDD in Environment 1 and 35.8 GDD in Environment 3. Dominance was the main source of variation. Mol7 contributed alleles for early silking for gTL in both regions. QTL for ASL were identified on chromosomes 3 and 5 with major dominance variation in environment 1 and on chromosome 4 with primarily additive variation in environment 2. Single marker analysis identified the regions with large effects on chromosome 1 and 8.

## DISCUSSION


#### Abstract

Comparison of Results in Individual Environments and Previous Studies Comparing the results from the three experiments, QTL for 2ECB were located on chromosomes 2 and 9 in all three environments. Chromosomes 1 and 10 were identified in two environments (Env. 1 and 2). Chromosomes 3, 4, 6, 7 and 8 were detected only in one of the three environments. For QTL identified in more than one environment, the defining interval varied to some degree. For example, QTL for 2ECB identified on chromosome 2 were marked by UMC34 and UMC131 in Environment 1, by AGP2 and NPI565 in Environment 2, and by UMC53 and UMC78 in Environment 3. The markers available provided an indication for the approximate location. These observations implied evaluation in several environments provided a more reliable estimate for QTL location.

QTL for 2ECB were located in a previous study in an $F_{2}$ population derived from single cross B73 (susceptible) x B52 (resistant) (Schön et al., 1993). Since the common resistant parental line $B 52$ was used and evaluation was conducted in two environments used in this study (Env. 1 and Env.2), comparison between the two studies may provide important information. Chromosomes 1, 2, 3, 7 and 10 were identified for QTL for 2ECB in the previous report. Results from the current study identified chromosomes 1, 2 and 10 in more than one environment, and chromosomes 3 and 7 in one of three environments. QTL on chromosome 8 were also detected in one environment. The most obvious difference, QTL on chromosome 9 were detected in each of three environments in this study. In both studies, alleles conferring resistance to $2 E C B$ for the regions with largest effects were derived from B52.

QTL for PT were analyzed by Schön et al. (1993) based on replicated progeny. Chromosome 1, 3 and 9 were identified to have gTL for PT. Only chromosome 1 was identified in both studies. In our results, chromosome 3


and 9 were not detected; however, chromosome 8 was detected in three environments, and chromosomes 4 and 7 were detected in one of the three environments. The difference might be caused by the variation in parental lines, different environments or/and experimental error. Beavis et al. (1991) reported 11 QTL for plant height in four maize populations. Among 10 chromosomes, only chromosomes 2 and 4 were not detected for plant height QTL. However, no chromosome was detected in all populations. Chromosomes 3 and 9 were detected in two populations.

Several mutants for plant height have been located on the published maize genetic linkage map (Coe et al., 1988). In this linkage map, information from different studies, including translocation studies, RFLP mapping and morphological marker location were integrated and relative positions of different categories of markers can be derived from the map. D8 (dwarf plant) locus was located on chromosome 1 near the probe identifying plant height, BNL15.18. Another Sdwl (semi-dwarf) locus was located on chromosome 8 on the same arm containing UMC89 and NPI268, to which we located QTL for PT. This result suggested that genes controlling the same traits at different levels could be located on the same chromosomes at the same loci (Robertson, 1985).

Chromosome 8 was identified for ATS and ASL in two environments. Chromosome 1 was identified for ASL in two environments. The regions identified in two environments tended to have larger LOD scores and contributed more to the trait phenotypes. Mol7 contributed alleles for early flowering in these regions.

The results over environments indicated that the regions with larger effects were detected in more than one environment and the location of QTL was identified with repeatability in this experiment. The regions detected in one environment tended to have relatively smaller effects. There are several explanations for identification in one environment. It could be
caused by genetic reason only, i.e., different QTL function in different environments. It was also possibly due to the requirement of accurate measurement of the trait for good differentiation of the genetic classes. The possibilities were discussed by Jarboe (1993).

## Gene Action and Estimation of Dominance in $F_{2}$ Population

Summarizing the information for all traits in all environments, additive variation was detected more in 2ECB tunneling, PT and ET, and dominant variation was detected more orten for ATS and ASL. This was consistent with estimates based on whole-genome biometric methods (Hallauer et al., 1988). Since an $F_{2}$ population was sampled, maximum linkage disequilibrium might lead to an overestimate of dominant effect (Gardner et al., 1953; Gardner, 1963). Stuber and Edwards (1987) suggested random mating the $F_{2}$ generation to reduce the bias caused by linkage disequilibrium.

Possible epistatic effects in three different environments varied severely. Epistasis among regions with QTL for 2 ECB was in the positive direction in Env. 1 and negative direction in Env. 3. Epistasis was not detected in Env. 2. The result was expected since different regions were detected in three environments. The estimate of epistasis could be due to true interaction among loci, various errors in the experiment, and bias.


#### Abstract

Comparison between Interval Mapping and Single Marker Analysis Interval mapping and single marker analysis gave similar results in this study; almost all regions identified by the two procedures were the same. When several closely linked markers were detected by single marker analysis, the probability values for the linked markers were at the same significance level. Two of the markers would be the loci to specify the interval for $Q T L$ in interval mapping. Alternate markers might be


identified in different environments; however, the loci would be in the closely linked region. For example, QTL for 2ECB on Chromosome 1 in Env. 2 were indicated by BNL15.18-UMC128 in interval mapping. Single marker analysis detected three linked UMC128, UMC23 and UMC33 in this region; however, UMC128 had the lowest probability and was closest to the gene(s) for 2ECB. Especially when the probes were evenly distributed along the chromosome and had high density (less than 10 cM between pairs of markers), the results from two procedures should be the same. Single marker analysis might provide useful information in two ways: 1) When interval mapping identified a region flanked by two distant markers, (e.g. interval UMC64PIO20.0075 on chromosome 10), single marker analysis might only detect the marker closer to the gene(s) for the trait and exclude a large region from the interval; and 2) Variation caused hy additive and dominant effects could be tested. Estimation of variation might contribute direct information to breeders. Interval mapping only estimated additive and dominant effect values. In general, first degree statistics (e.g. means) were not as tolerant to bias as second degree statistics (e. g. variance). Bias could be caused by recombination values and linkage disequilibrium. Additive and dominant variation could be tested and provide a significant test for the variation in the population.

## Comparison between RFLP Mapping and Translocation Mapping for ECB

In the translocation study conducted by Onukogu et al. (1978), B52 was the resistant parent. Two susceptible inbreds were Oh43 and W182E. The infestation level was similar to the one used in the current study (500 egge/plant). $B 52$ was crossed with translocation stocks in M14 background. The semisterile $F_{1}$ were crossed to the two susceptible inbreds. Progeny were evaluated in two years. Resistant factors to 2ECB were located on long arms of chromosomes 1, 2, 4 and 8 , short arms of 1, 3 and 5. All
chromosome arms detected by translocations were identified by RFLP mapping in one or more environments. The region on chromosome 10 which dexived resistance from Mol7 in RFLP mapping was not detected by translocations. One region on chromosome 9 showed high level of contribution to trait expression, and was not detected in the translocation study or by Schöne et al. (1993). The resistance seemed to be either expressed only in the genetic background of $\mathrm{B} 52 \mathrm{xMo17}$ or some other unknown mechanism.


#### Abstract

Correlation among Traits and Relationship between QTL Location Correlation among traits has been explained by two possible mechanisms, linkage between genes controlling correlated traits and pleiotropy (Falconer, 1989). Accurate location of QTL for traits may provide information regarding the biological basis of correlation among traits. As expected, the highest correlations were found between PT and ET, and between ATS and ASL. QTL for PT and ET were located to the same regions on chromosomes 1, 7 and 8 in Env. 1, on chromosome 1 and 8 in the other two environments. Chromosome 8 was detected containing QTL for ATS and ASL in all environments. The results provided additional proof for the two proposed correlation mechanisms, either the same loci controlling the correlated traits or the loci controlling the traits were linked; however, the current level of probe density did not allow differentiation between the two possibilities for these two pairs of traits.


Table 1. Loci with segregation ratios deviating from the expected values ${ }^{\lambda}$

| Chr. | Loci | Genotypic Classes <br> AA AB BB |  |  | Allele Frequency <br> A B |  | $\mathrm{P}\left(>\mathrm{x}^{2}\right)^{B}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | No. of $\mathrm{F}_{2} 3$ lines |  |  |  |  |  |
| 2 | UMC88 | 28 | 84 | 47 | . 43 | . 57 | . 04 |
|  | AGP2 | 29 | 100 | 38 | . 47 | . 53 | . 02 |
|  | UMC98 | 26 | 84 | 50 | . 43 | . 57 | . 02 |
| 4 | UMC158 | 32 | 100 | 35 | . 49 | . 51 | . 04 |
|  | PIO10.0025 | 31 | 108 | 30 | . 50 | . 50 | . 002 |
|  | NPI203 | 30 | 101 | 32 | . 49 | . 51 | . 01 |
| 5 | pzmISU033 | 45 | 98 | 26 | . 56 | . 44 | . 02 |
|  | UMC166 | 42 | 96 | 26 | . 55 | . 45 | . 02 |
|  | BNL5.71 | 29 | 97 | 31 | . 49 | . 51 | . 02 |
|  | BNL8. 33 | 33 | 103 | 33 | . 47 | . 53 | . 02 |
| 6 | UMC85 | 28 | 96 | 44 | . 45 | . 55 | . 04 |
|  | Pl1 | 32 | 75 | 54 | . 43 | . 57 | . 03 |
| 7 | BNL15.40 | 30 | 102 | 37 | . 48 | . 52 | . 02 |
|  | DEK326 | 33 | 101 | 34 | . 50 | . 50 | . 03 |
|  | BNL13.24 | 31 | 103 | 35 | . 49 | . 51 | . 02 |
|  | BNL14.07 | 29 | 97 | 41 | . 46 | . 54 | . 05 |
|  | UMC110 | 30 | 103 | 35 | . 49 | . 51 | . 01 |
|  | UMC116 | 31 | 99 | 36 | . 48 | . 52 | . 03 |
|  | BNL15.21 | 33 | 100 | 35 | . 49 | . 51 | . 05 |
| 8 | BNL8. 26 | 54 | 79 | 32 | . 57 | . 43 | . 04 |
|  | NPI220 | 29 | 105 | 28 | . 50 | . 50 | . 00 |
| 9 | PIO10.0005 | 33 | 75 | 54 | . 44 | 4.54 | . 05 |
|  | UMC20 | 37 | 103 | 28 | . 53 | - . 47 | . 01 |

${ }^{A} A A, A B$ and $B B$ represented homozygous for Mo17, heterozygous, and homozygous for B 52 , respectively. $A$ and $B$ indicated gene frequency for allele(s) from Mol7 and B52, respectively.
${ }^{B}$ Probability of $x^{2}>x_{\alpha}$

Table 2. Summary Statistics and Normality Tests of the Tunnelling Length of 2ECB

| Environment | Mean <br> (inches) | Range <br> (inches) | C.V.A (\%) | Prob<W ${ }^{\text {B }}$ |
| :--- | :---: | :---: | :---: | :---: |
|  |  |  |  |  |
| Env. 1 | 24 | $14-40$ | 22 | 0.08 |
| Env. 2 | 17 | $6-29$ | 30 | 0.02 |
| Env. 3 | 7 | $3-15$ | 37 | 0.001 |
| Combined Data | 16 | $9-25$ | 21 | 0.05 |

A coefficient of variation
${ }^{\mathbf{B}}$ Probability of $W<W_{\alpha}$

Table 3. Variation Analysis and Heritability Estimates for 2ECB Tunnelling on $\mathrm{F}_{2: 3}$ Mean Basis

| Environment | $\mathrm{h}^{2+}$ | $\sigma_{8}^{2}$ | $\sigma_{8 q}^{2}$ | $\sigma_{s}^{2}$ |
| :---: | :---: | :---: | :---: | ---: |
|  |  |  |  |  |
| Env. 1 | $62.3(48.3-72.5)$ | $18.57^{* * *}$ |  | 22.48 |
| Env. 2 | $68.5(58.4-77.9)$ | $20.87^{* * *}$ |  | 19.19 |
| Env. 3 | $47.2(27.8-61.7)$ | $3.49^{* *}$ |  | 7.73 |
| Combined Data | $62.2(50.6-71.4)$ | $7.99^{* *}$ | $6.33^{* *}$ | 16.47 |
|  |  |  |  |  |

** significant at 0.01 level

+ confidence interval for $h^{2}$ estimate in the brackets

Table 4. Regions for ECB Tunnelling Identified by Interval Mapping

| Chr | Interval | Summary Scan |  | Multiple Loci Analysis |  |  |  | GA ${ }^{\mathbf{A}}$ | $L P^{8}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | peak <br> LOD | $\mathrm{R}^{2}$ (\%) | $\begin{gathered} \text { Length } \\ (C M) \end{gathered}$ | $\begin{gathered} \text { Position } \\ \text { (CM) } \end{gathered}$ | Gene E <br> (i | $\begin{aligned} & \text { ects }^{\mathrm{F}} \\ & \text { Don } \\ & \text { hes) } \end{aligned}$ |  |  |
| Env. 1 |  |  |  |  |  |  |  |  |  |
| 1 | BNL8.29-BNL15.18 | 5.2 | 18.2 | 29.9 | 27 | $-2.3$ | - 2.0 | A/D | B52 |
| 2 | UMC34-UMC131 | 4.5 | 13.9 | 14.8 | 14 | - 1.8 | - 1.6 | D | B52 |
| 9 | UMC114-BNL8.17 | 3.7 | 10.8 | 11.9 | 0 | - 1.9 | $-2.6$ | A | B52 |
| 10 | NPI232-NPI287 | 3.1 | 10.8 | $\begin{aligned} & 17.5 \\ & R^{2}=36.0^{D} \end{aligned}$ | 13 | 1.3 | 3.2 | D | Mol7 |
|  | Sum | 16.5 |  | $\mathrm{R}^{2}=36.0^{\mathrm{D}}$ |  | LOD=13.3E |  |  |  |
| Env. 2 ( |  |  |  |  |  |  |  |  |  |
| 1 | BNL15.18-UMC128 | 5.0 | 15.2 | 25.4 | 24 | $-1.8$ | - 1.8 | D/A | B52 |
| 2 | AGP2-NPI565 | 2.7 | 8.0 | 2.9 | 0 | 1.9 | 1.4 | D | Mo17 |
| 3 | BNL5.37-UMC26 | 2.7 | 8.6 | 11.5 | 2 | - 1.6 | $-3.6$ | A | B52 |
| 4 | BNL15.07-NPI203 | 3.2 | 11.4 | 20.2 | 12 | 1.3 | 1.6 | A/D | Mol7 |
| 8 | BNL9.08-BNL9.44 | 5.4 | 15.3 | 3.5 | 0 | - 2.2 | - 1.0 | A | B52 |
| 9 | UMC20-UMC153 | 3.9 | 11.2 | 1.20 |  | - 2.1 | $-1.0$ | A/D | B52 |
| 10 | UMC64-PIO20.0075 | $\begin{array}{rr}2.8 & 15.2 \\ 25.7 & \end{array}$ | 15.2 |  |  |  | E.6 | A | Mol7 |
|  | Sum |  |  | $\begin{aligned} & 81.0 \\ & R^{2}=58.48^{10} \end{aligned}$ |  | LOD=25.1 ${ }^{\text {E }}$ |  |  |  |
| Env. 3 |  |  |  |  |  |  |  |  |  |
| 2 | UMC53-UMC78 | 2.8 | 12.3 | 33.5 | 20 |  | 0.4 | D/A | B52 |
| 7 | UMC110-BNL15.21 | 3.9 | 13.1 | $16.4$ | 4 | - 1.2 | - 1.6 | A | B52 |
| 9 | UMC81-UMC20 | 3.1 | 9.0 | $1.2$ | 0 | $-0.9$ | $-1.6$ | A/D | B52 |
|  | Sum | 9.8 |  | $\mathbf{R 2}=54$. | . $5 \%^{\text {D }}$ | LOD $=18$ | $4^{E}$ |  |  |

A Results from significance test by single marker analysis, at the significance level of $\alpha=0.001$, $A$ indicated significant additive variation, D indicated significant dominant variation, A/D indicated significant additive and dominant variation while additive variation had lower probability than dominance variation, $D / A$ indicated significant additive and dominant variation while dominance variation had lower probability than dominance variation, and - indicated no significant additive and dominance variation was detected.
a LP indicated the parent contributing alleles conferring lower value for the trait
c Position of QTL relative to the loci defining the interval, denoted by the distance from the loci on the left in the interval
D Determination factor from multiple loci analysis.
E LOD score from multiple loci analysis.
F Estimates of gene effects from multiple loci analysis. Add. and Dom. indicated additive and dominance effects, respectively.

Table 5. Summary Statistics and Normality Tests for Morphological Traits

| Trait Env. | Mean | Range | c.v.^ | Prob< $\mathrm{W}^{\text {B }}$ |
| :---: | :---: | :---: | :---: | :---: |
| cm |  |  |  |  |
| 1 | 263 | 205-305 | 7.4 | 0.36 |
| PT 2 | 232 | 70-257 | 9.4 | 0.86 |
| $3$ | 207 | 155-284 | 11.6 | 0.001 |
| Combined |  | 160-277 |  |  |
| 1 cm |  |  |  |  |
| 1 | 118 | 86-156 | 12.0 | 0.16 |
| ET 2 | 83 | 28-116 | 14.2 | 0.08 |
| 3 | 88 | 41-185 | 18.8 | $<0.01$ |
| Combined | 89 | 52-136 | 17.3 | 0.82 |
| 1 days ${ }^{\text {d }}$ (19-31 310.10 |  |  |  |  |
|  |  |  |  |  |
| TS 3 | 43 | 37- 50 | 5.8 | 0.03 |
| Combined | 34 | 28- 39 | 5.9 | 0.25 |
| 1 days 10.1 |  |  |  |  |
| 1 | 29 | 22-40 | 10.1 | 0.72 |
| SL 3 | 46 | 38-59 | 7.2 | 0.21 |
| Combined | 37 | 30-48 | 8.0 | 0.83 |
| 1 |  |  |  |  |
|  |  |  |  |  |
| ATS 3 | 1210 | 1133-1336 | 3.1 | 0.16 |
| Combined | 1053 | 950-1134 | 2.9 | 0.86 |
| GDD |  |  |  |  |
| 1 | 944 | 821-1151 | 4.8 | 0.08 |
| ASL 3 | 1267 | 1139-1496 | 5.1 | 0.002 |
| Combined | 1105 | 996-1285 | 4.5 | 0.36 |

[^0]Table 6. Variation Analysis and Heritability Estimates for Morphological Traits

| Trait Env. | $\mathrm{h}^{2}$ | $\sigma_{8}{ }^{2}$ | $\sigma_{\mathrm{go}}{ }^{2}$ | $\sigma_{\mathrm{s}}{ }^{2}$ |
| :---: | :---: | :---: | :---: | :---: |
| 1 | 81.9 (75.2-86.8) | $313.5{ }^{* *}$ |  | 138.2 |
| PT 2 | 68.2 (56.3-76.8) | 394.7** |  | 368.4 |
| 3 | 61.2 (46.8-71.7) | 292.4** |  | 370.6 |
| Combined | 74.2 (66.2-80.5) | 306.1* | 96.9** | 446.5 |
| 1 | 83.4 (77.2-87.9) | 167.4** |  | 66.7 |
| ET 2 | 70.2 (59.2-78.3) | 97.7***********) |  | 82.9 |
| 3 | 62.1 (48.0-72.4) | 170.0** |  | 207.6 |
| Combined | 79.5 (73.2-84.5) | $112.2 *$ | 38.7 | 686.7 |
| 1 | 72.2 (61.8-79.7) | 3.2*** |  | 2.5 |
| TS 3 | 62.2 (72.4-48.1) | 3.7*** |  | 4.6 |
| Combined | 70.8 (59.9-78.7) | $2.9{ }^{*}$ | $0.6 *$ | 3.5 |
| 1 | 80.6 (73.3-86.9) | 6.8** |  | 3.3 |
| SL 3 | 63.2 (50.0-73.2) | 8.0 . |  | 9.3 |
| Combined | 83.0 (76.7-87.6) | $7.5{ }^{\circ *}$ | 0 | 6.3 |
| 1 | 69.7 (58.4-77.9) | 754.4**********) |  | 655.9 |
| ATS 3 | 58.2 (42.6-69.5) |  |  | 1193.7 |
| Combined | 68.7 (59.2-76.0) | $653.9^{\circ *}$ | 136.4* | 919.8 |
| 1 | 79.6 (72.1-85.2) | 1600.7*** |  | 818.3 |
| ASL 3 | 54.6 (37.7-66.9) | 2263.1** |  | 3766.8 |
| Combined | 79.5 (71.8-85.0) | 1968.4** | 0 | 1070.5 |

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* significant at 0.05 level
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** significant at 0.01 level

Table 7. Phenotypic and Genetic Correlation coefficients among Traits on the Basis of Entry Means

|  | PT | ET | 2ECB | TS | SL | ATS | ASL |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| PT | - | 0.73 | 0.11 | 0.26 | 0.25 | 0.27 | 0.24 |
| ET | $\begin{gathered} 0.86 \\ (0.0001) \end{gathered}$ | - | 0.11 | 0.23 | 0.24 | 0.23 | 0.24 |
| CB | $\begin{gathered} 0.11 \\ (0.1628) \end{gathered}$ | $\begin{gathered} 0.12 \\ (0.1319) \end{gathered}$ | - | -0.004 | 0.13 | 0.01 | 0.14 |
| TS | $\begin{gathered} 0.26 \\ (0.0010) \end{gathered}$ | $\begin{gathered} 0.30 \\ (0.0002) \end{gathered}$ | $\begin{gathered} 0.01 \\ (0.8991) \end{gathered}$ | - | 0.79 | 0.99 | 0.78 |
| SL | $\begin{gathered} 0.25 \\ (0.0019) \end{gathered}$ | $\begin{gathered} 0.22 \\ (0.0064) \end{gathered}$ | $\begin{gathered} 0.15 \\ (0.0660) \end{gathered}$ | $\begin{gathered} 0.79 \\ (0.0001) \end{gathered}$ | - | 0.78 | 0.99 |
| ATS | $\begin{gathered} 0.27 \\ (0.0005) \end{gathered}$ | $\begin{gathered} 0.31 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.01 \\ (0.8759) \end{gathered}$ | $\begin{gathered} 0.98 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.78 \\ (0.0001) \end{gathered}$ | - | 0.77 |
| ASL | $\begin{gathered} 0.23 \\ (0.0032) \end{gathered}$ | $\begin{gathered} 0.20 \\ (0.0109) \end{gathered}$ | $\begin{gathered} 0.14 \\ (0.0862) \end{gathered}$ | $\begin{gathered} 0.78 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.99 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.77 \\ (0.0001) \end{gathered}$ | - |

A Numbers above the diagonal were genetic correlation coefficients, numbers under the diagonal were phenotypic correlation coefficients. Numbers in brackets were the probability values for significance test for correlation coefficients.

Table 8. Phenotypic Correlation Coefficients among Traits

|  | ET | CB | TS | SL | ATS | ASL |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Env. 1 |  |  |  |  |  |
| PT | $\begin{gathered} 0.77 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.14 \\ (0.0145) \end{gathered}$ | $\begin{gathered} 0.22 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.31 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.22 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.28 \\ (0.0001) \end{gathered}$ |
| ET |  | $\begin{gathered} 0.11 \\ (0.0438) \end{gathered}$ | $\begin{gathered} 0.24 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.21 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.24 \\ (0.0010) \end{gathered}$ | $\begin{gathered} 0.18 \\ (0.0001) \end{gathered}$ |
| CB |  |  | $\begin{gathered} 0.06 \\ (0.2573) \end{gathered}$ | $\begin{gathered} 0.11 \\ (0.0442) \end{gathered}$ | $\begin{gathered} 0.06 \\ (0.2729) \end{gathered}$ | $\begin{gathered} 0.10 \\ (0.0707) \end{gathered}$ |
| TS |  |  |  | $\begin{gathered} 0.71 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.97 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.68 \\ (0.0001) \end{gathered}$ |
| SL |  |  |  |  | $\begin{gathered} 0.69 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.99 \\ (0.0001) \end{gathered}$ |
| ATS |  |  |  |  |  | $\begin{gathered} 0.67 \\ (0.0001) \end{gathered}$ |
|  |  | Env. 2 |  |  |  |  |
| PT | $\begin{gathered} 0.81 \\ (0.0001) \end{gathered}$ | $\begin{gathered} -0.15 \\ (0.0064) \\ 0.08 \\ (0.1601) \end{gathered}$ |  |  |  |  |
| ET |  |  |  |  |  |  |
|  | Env. 3 |  |  |  |  |  |
| PT | $\begin{gathered} 0.72 \\ (0.0001) \end{gathered}$ | $\begin{gathered} -0.32 \\ (0.0001) \end{gathered}$ | $\begin{array}{r} 0.24 \\ (0.0024) \end{array}$ | $\begin{array}{r} 0.11 \\ (0.1875) \end{array}$ | $\begin{gathered} 0.23 \\ (0.0027) \end{gathered}$ | $\begin{gathered} 0.10 \\ (0.2240) \end{gathered}$ |
| ET |  | $\begin{gathered} -0.17 \\ (0.0324) \end{gathered}$ | $\begin{gathered} 0.35 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.15 \\ (0.0633) \end{gathered}$ | $\begin{array}{r} 0.33 \\ (0.0001) \end{array}$ | $\begin{array}{r} 0.12 \\ (0.1348) \end{array}$ |
| CB |  |  | $\begin{array}{r} -0.11 \\ (0.1552) \end{array}$ | $\begin{gathered} -0.04 \\ (0.5828) \end{gathered}$ | $\begin{gathered} -0.09 \\ (0.2869) \end{gathered}$ | $\begin{gathered} 0.05 \\ (0.5248) \end{gathered}$ |
| TS |  |  | (0.15 | $\begin{gathered} 0.68 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.98 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.65 \\ (0.0001) \end{gathered}$ |
| SL |  |  |  |  | $\begin{gathered} 0.66 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.97 \\ (0.0001) \end{gathered}$ |
| ATS |  |  |  |  |  | $\begin{aligned} & 0.66 \\ & (0.0001) \end{aligned}$ |

Table 9. Regions for Plant Height and Ear Height Identified by Interval Mapping

| Chr | Interval | Summary Scan |  | Multiple Loci Analysie |  |  |  | GA ${ }^{\text {A }}$ | $L P^{\text {B }}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | peak LOD | $\mathrm{R}^{2}$ (\%) | Length (cM) | Position ${ }^{\mathbf{c}}$ (cM) | Gene Effects ${ }^{\text {F }}$ |  |  |  |
|  |  |  |  |  |  | Add. | Dom. |  |  |
|  |  |  |  |  |  | ( cm ) |  |  |  |
|  |  |  | PT. |  |  |  |  |  |  |
| 1 | BNL15.18-UMC128 | 7.4 | 27.3 | 25.4 | 12 | -13.6 | 1.8 | $D / A$ | B52 |
| 6 | UMC21-P11 | 2.9 | 10.1 | 26.7 | 7 | - 5.2 | 11.6 | D | B52 |
| 8 | NPI268-UMC89 | 4.7 | 16.3 | $25.5$ | 16 | $9.0$ | 10.2 | A/D | Mo17 |
|  | Sum | $15.0$ |  | R2=54. |  | $L O D=18.0^{E}$ |  |  |  |
|  |  |  | PT |  |  |  |  |  |  |
| 1 | BNL15.18-UMC128 | 5.2 | 18.5 | 25.4 | 8 | -11.3 | 2.4 | $D / A$ | B52 |
| 3 | UMC50-UMC121 | 2.5 | 16.5 | 56.2 | 24 | -10.6 | 17.4 | - | B52 |
| 8 | NPI268-UMC89 | 5.0 | 23.1 | 25.5 | 23 | 13.4 | 6.4 | A/D | Mol7 |
|  | Sum | 12.7 |  | R2=58. |  | LOD=17.5 ${ }^{\text {E }}$ |  |  |  |
|  |  |  | $\mathrm{PT}_{2}$ |  |  |  |  |  |  |
| 1 | BNL15.18-UMC128 | 8.9 | 32.2 | 25.4 | 10 | -14.9 | 7.2 | $D / A$ | B52 |
| 8 | NPI268-UMC89 | 5.6 | 19.2 | $25.5$ | 22 | $7.8$ | 19.2 | A | Mo17 |
|  | Sum | 14.5 |  | $\mathrm{R}^{2}=49.0$ |  | $L O D=15.1^{E}$ |  |  |  |
|  |  |  |  |  |  |  |  |  |  |
| 1 | BNL15.18-UMC128 | 3.3 | 11.4 | 25.4 | 5 | $-6.4$ | -0.6 | $D / A$ | B52 |
| 6 | PIO10.0016-NPI560 | 3.0 | 9.7 | 15.9 | 14 | $-7.0$ | -0.02 | A | B52 |
| 7 | BNL15.21-UMC116 | 4.1 | 13.4 | 9.1 | 2 | 7.8 | 8.6 | A | Mo17 |
| 8 | NPI268-UMC89 | $3.3$ | 14.8 | 25.5 | 16 | $5.9$ | 10.0 | A | Mo17 |
|  | Sum | $13.7$ |  | $\mathrm{R}^{2}=4$ | . 2\% ${ }^{\text {D }}$ | LOD $=15$ | $9^{E}$ |  |  |
|  |  |  | ET. |  |  |  |  |  |  |
| 1 | BNL15.18-UMC128 | 6.4 | 21.5 | 25.4 | 4 | - 5.9 | -2.6 | A/D | B52 |
| 1 | BNL12.06-BNL5.62 | 3.2 | 18.3 | 47.3 | 35 | $-2.5$ | -10.6 | A | B52 |
| 8 | NPI268-UMC89 | $4.3$ | $15.8$ |  | $20$ | $4.8$ | 8.0 | A | Mo17 |
|  | Sum | $13.9$ | $55 .$ | $R^{2}=4$ | $1.4 \%^{\mathrm{D}}$ | LOD=12. |  |  |  |
|  |  |  | ETe |  |  |  |  |  |  |
| 1 | BNL8.29-UMC128 | 5.8 | 22.8 | 29.9 | 26 | -10.3 | $-8.0$ | D/A | B52 |
| 8 | NPI268-UMC89 | 3.9 | 13.8 | 25.5 | 24 | 5.0 | 13.4 | A | Mol7 |
|  | Sum | 9.7 | 36.6 | R2 $=3$ | .8\% ${ }^{\text {D }}$ | LOD=9.9 ${ }^{\text {E }}$ |  |  |  |

${ }^{\wedge}$ Results from significance test by single marker analysis, at the significance level of $\alpha=0.001$, $A$ indicated significant additive variation, $D$ indicated significant dominant variation, $A / D$ indicated significant additive and dominant variation while additive variation had lower probability than dominance variation, $D / A$ indicated significant additive and dominant variation while dominance variation had lower probability than dominance variation, and - indicated no significant additive and dominance variation was detected.
B LP indicated the parent contributing alleles conferring lower value for the trait
c position of QTL relative to the loci defining the interval, denoted by the distance from the loci on the left in the interval
D Determination factor from multiple loci analysis.
E LOD score from multiple loci analysis.
F Estimates of gene effects from multiple loci analysis. Add. and Dom. indicated additive and dominance effects, respectively.

Table 10. Regions for $G D D$ to Anthesis and Silk Emergence Identified by Interval Mapping

| Chr. | Interval | Summary Scan |  | Multiple Loci Analysis |  |  |  | GA ${ }^{\text {A }}$ | $L \mathrm{P}^{\text {B }}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | peak <br> LOD | $R^{2}$ (\%) | Length <br> (CM) | $\begin{aligned} & \text { Positi } \\ & \text { (cM) } \end{aligned}$ | $\frac{\text { Gene E }}{\text { Add. }}$ | $t \mathrm{~B}^{\mathrm{F}}$ <br> Dom |  |  |
| 8 | UMC89-BNL8. 26 | 8.2 | ATS, Env. 1. GDD |  |  |  |  |  |  |
|  |  |  | 22.4 | 2.3 | 0 | 21.4 | $-4.2$ | D/A | Mo17 |
|  |  |  | ATS, E | GDD |  |  |  |  |  |
| 8 | NPI268-UMC89 | 8.1 | 26.1 | 25.6 | 12 | 26.2 | 3.4 | $D / A$ | Mo17 |
|  |  |  | ASL, E | GDD |  |  |  |  |  |
| 1 | NPI234-UMC11 | 3.1 | 9.0 | 1.3 | 0 | 24.0 | -19.6 | D | Mo17 |
| 3 | UMC60-UMC164 | 2.7 | 7.8 | 10.3 | 0 | -12.1 | $-1.4$ | D | B52 |
| 5 | BNL7.71-BNL5.71 | 3.7 | 14.1 | 16.4 | 10 | 21.7 | -17.8 | D | Mol7 |
| 8 | UMC89-BNL8. 26 | 9.6 | 26.1 | 2.2 | 0 | 29.9 | 10.4 | D/A | Mo17 |
|  | Sum | 19.1 | 57.0 | $\mathrm{R}^{2}=52.0 \%^{\text {D }}$ |  | $L O D=21.9^{\mathrm{E}}$ |  | D/A |  |
| ASL, Env. 3, GDD |  |  |  |  |  |  |  |  |  |
| 1 | UMC157-NPI234 | 4.1 | 12.8 | 17.7 | 13 | 35.8 | -32.0 | D/A | Mo17 |
| 4 | NPI203-PIO10.0025 | 2.8 | 9.5 | 6.3 | 4 | -18.9 | -47.0 | A | B52 |
| $\varepsilon$ | NPI268-UMC89 | 7.8 | $36.9$ | 25.5 | 16 | $52.7$ | -43.0 | D | Mo17 |
|  | Sum | 14.7 | 59.2 | $\mathrm{R}^{2}=55$ | $1 \%^{D}$ | LOD=1 |  |  |  |

A Results from significance test by single marker analysis, at the significance level of $\alpha=0.001$, $A$ indicated significant additive variation, $D$ indicated significant dominant variation, $A / D$ indicated significant additive and dominant variation while additive variation had lower probability than dominance variation, $D / A$ indicated significant additive and dominant variation while dominance variation had lower probability than dominance variation, and - indicated no significant additive and dominance variation was detected.
I IP indicated the parent contributing alleles conferring lower value for the trait
c Position of QTL relative to the loci defining the interval, denoted by the distance from the loci on the left in the interval
D Determination factor from multiple loci analysis.
E LOD score from multiple loci analysis.
F Estimates of gene effects from multiple loci analysis. Add. and Dom. indicated additive and dominarice effects, respectively.


Figure 1. Genomic Composition for Population B52 $\times$ Mol7. $x$ axis indicated the percentage of 852 , Mo17 and B52 x Mo17 components. Y axis indicated the number of individuals.



Figure 2. RFLP Linkage Map of Population B52 x Mol7

Genetic length measured $=1504 \mathrm{cM} \quad$ Number of loci=113 Average spacing between loci=13 cM
cl-c10 correspond to chromosome 1 to 10 in maize genome. The vertical lines represent genetic length measured by RFLP markers. Horizontal dark bars along the genetic length indicated RFLP markers. Names of RFLP markers appear on the left of each chromosome. Genetic distance between RFLPs are at the right of each chromosome.

* RFLP markers producing segregation ratio with deviation from 1:2:1 ratio ( $\alpha=0.05$ ).

C1


C2


C3


C4


Figure 3. Plot of LOD Score for 2ECB Tunneling Detected for Each Environment.
The X axis represents the chromosome with vertical bars indicating the distribution of RFLP loci. The $Y$ axis is the scale of LOD scores.


C7


C8


C9



Env. 1
_-_- Env. 2
———Env. 3

C1


C2


C3


C4


Figure 4. Plot of LOD Score for Plant Height Detected for Each Environment.
The $X$ axis represents the chromosome with vertical bars indicating the distribution of RFLP loci. The $Y$ axis is the scale of LOD scores.

C5


C8


C7


C8


C9


C10

___ Env. 1
———Env. 2
---- Env. 3

C1


C2


C3


C4


Figure 5. Plot of LOD Score for Ear Height Detected for Each Environment.
The $X$ axis represents the chromosome with vertical bars indicating the distribution of RFLP loci. The $Y$ axis is the scale of LOD scores.


C8
C7


C8


$\mathrm{C} 10 \underset{\mathrm{dan}}{\mathrm{dan}}$

Env. 1
Env. 2
Env. 3

C1


C2

$C 3$


C4


C5


Figure 6. Plot of LOD Score for Anthesis (GDD) Detected for Each Environment.
The $X$ axis represents the chromosome with vertical bars indicating the distribution of RFLP loci. The $Y$ axis is the scale of LOD scores.






Env. 1
—_ Env. 3

C1


C2


C3


C4


C5


Figure 7. Plot of LOD Score for Silk Emergence date (GDD) Detected for Each Environment.
The $X$ axis represents the chromosome with vertical bars indicating the distribution of RELP loci. The $Y$ axis is the scale of LOD scores.


C7


C8



C10

$\ldots$ Env. 1


Env. 3

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PAPER II. LOCATION AND ANALYSIS OF CHROMOSOME REGIONS AFFECTING MORPHOLOGICAL TRAITS

IN A MAIZE POPULATION

ABSTRACT

Restriction fragment length polymorphisms (RFLPs) were used to locate quantitative trait loci (QTL) for morphological traits in maize population B52 x Mol7. One hundred and thirteen genomic and CDNA clones were used to construct a linkage map. One hundred and fifty $F_{2: 3}$ lines were scored for RFLP patterns and evaluated for the performance of the traits in two environments, Ames 1990 and 1991. Water stress was evident in 1991 in contrary to the normal condition in 1990. Interval mapping and aingle marker analysis were used to locate QTL.

Putative QTL for anthesis (GDD) were detected on chromosomes 2, 3 and 8 in both environments, and on chromosomes 1,5 and 10 in one of the two environments. Chromosomes 1 and 8 were identified for QTL for silking date (GDD) in both environments and chromosomes $2,5,6$ and 9 were detected in one environment. For these two highly correlated traits (r>0.7), the two important regions on chromosomes 1 and 8 were detected by the same intervals. Putative QTL for plant and ear height were identified on chromosome 1 in both environments. Chromosomes 3 and 8 were identified for plant height in one of the two environments, and chromosomes 5, 7 and 8 were identified for ear height in one of the two environments.

Genotype by environment interaction was detected only for anthesis; however, large discrepancies between LOD scores in two environments were found for silk emergence, plant height and ear height. The lack of detection of these difference through whole-genome biometrical methods might be due to cancellation of interaction in different directions among regions.

Dominance variation was detected more often than additive variation, which might reflect the inheritance of the traits or bias of estimates due to linkage disequilibrium in $F_{2}$ populations.

Restriction Fragment Length Polymorphisms (RFLPs) have been proposed as a means of dissecting quantitative trait loci [ (QTL), Burr et al., 1983]. Quantitative genetics has provided valuable information to breeding programs by using statistical methods to analyze the pooled effects of multiple loci. RFLPs provide abundant genetic markers for analysis of quantitative traits and should provide novel information regarding QTL (Tanksley et al., 1989).

Several attempts have been made to locate QTL for agronomic traits in maize and other crop species. QTL for plant height, ear height and silk emergence date were located to the vicinity of isozyme markers. Digenic epistasis was not detected. The type of gene action varied among regions for a given trait and among traits (Edwards et al., 1987). Results from this research indicated current analysis was adequate to detect QTL and provide information on an individual locus basis. QTL for 24 traits including morphological features and yield components were located with single marker analysis in two maize populations using isozyme markers (Stuber and Edwards, 1987 and Edwards et al., 1987). Abler et al. (1991) conducted a study with parental lines morphologically more similar than the parents used in previous studies. Sufficient variation in the $\mathbf{F}_{\mathbf{2}}$ population allowed QTL location with isozyme markers. This result is promising to breeders because the parents used were more similar to the populations used in practical breeding programs. Beavis et al. (1991) identified 11 regions for plant height in four maize populations by RFLP markers. The eleven regions represented eight of the ten chromosomes. Reiter et al. (1991) identified six regions for tolerance to low-phosphorus stress.

Paterson et al. (1991) identified 29 putative QTL for tomato fruit
traits in three environments. Four QTL were detected in three environments, 10 in two environments and the remainder only in one environment. In other studies conducted in more than one population, locations of QTL for the same trait varied among populations (Stuber and Edwards, 1987; Edwards et al., 1987; Beavis et al., 1991).

Performance of most economic traits are influenced by environmental factors. The cause of different performance of genotypes in varied environments may be genetic or environmental. Location of QTL by molecular markers further established the environmental contribution to quantitative trait performance. It is important to evaluate more populations over environments to add information towards the understanding of QTL action. Precipitation is one of the most important environmental factors affecting crop production. Water condition influences the physiological process of plant development. Detection of QTL for agronomic traits and morphological traits under different precipitation conditions may provide valuable information for plant breeders.

Plant stature and maturity are two important traits in maize improvement programs (Hallauer et al., 1988). The objectives of this study were: 1) to identify chromosome regions associated with morphological traits, including plant height, ear height and flowering date (growing degree days); 2) to analyze types of the gene action for these traits; and 3) to compare the estimates across two environments distinguished by normal and water stress conditions in 1990 and 1991, respectively.

## Population Development

Two inbred lines, $B 52$ and Mo17, were crossed and the hybrid was selfpollinated to produce the $F_{2}$ population for this experiment. An unselected sample of $150 F_{2}$ plants were self-pollinated to produce $F_{2: 3}$ families. Fifteen kernels from each $F_{2: 3}$ family were planted in the greenhouse for DNA sample collection. Equal amounts of leaf tissue were harvested from 8 to 15 plants for each family. Sib-mating was conducted among 20 plants per $F_{2: 3}$ family and an equal amount of seed from each plant was bulked as the seed supply of $F_{2: 3}$ lines for replicated trials.

## Experimental Design

The sample of $150 \mathrm{~F}_{2: 3}$ lines and six entries composed of balanced bulk of $F_{2: 3}$ seed were planted in a $12 \times 13$ rectangular Lattice Design for field evaluation with two replications in each of the two environments:

| Experiment Number | Environment | Environmental Code in <br> the dissertation |
| :---: | :---: | :---: |
| 00106 | Ames, 1990 | Env. 1 |
| 10106 | Ames, 1991 | Env. 2 |

Env. 1 reflected the normal growing conditions without water stress and Env. 2 was water stress condition (Appendix 11).

For a comparison of the growing conditions in the two seasons, Appendix 11 listed the average temperature, precipitation and accumulated heat units (GDD) on a monthly basis in 1990 and 1991 and the average over the last 30 years at the Agronomy Research Center at Ames, Iowa (Carlson and Lamkey, 1992, personal communication). Precipitation for 1990 was well above normal, with 20.23 inches of precipitation for June, July and August. The precipitation from May to August was 28.74 inches. On the other hand, the conditions in 1991 were quite different compared to normal. Early in the
season in 1991, the precipitation was much higher than normal, especially in April (9.17 inches in 1991 vs. 3.40 inches for normal and 2.00 in 1990); however, water stress occurred during the summer months in 1991. The total precipitation during May, June, July and August in 1991 was 15.76 inches compared to the normal of 16.82 inches. The total precipitation during June, July and August in 1991 was 9.57 inches compared to the normal of 12.45 inches. Including the precipitation in September, the precipitation was 11.93 inches in 1991 and 15.57 inches for normal years. Accompanied by the water stress, accumulation of heat units (GDD) was more dramatic from May to September in 1991 (Appendix 11). By September, 3218 GDD heat units were accumulated, about 270 higher than normal condition ( 2941 GDD). Heat unit accumulation was very close to normal conditions in 1990 (3053.5 GDD). The rate of GDD accumulation in 1990 very closely matched the normal condition on a per month basis. The rate of GDD accumulation was much higher than normal and in 1990.

Plots consisted of single rows 18 feet long with 2.5 feet between adjacent rows. Plots were machine-planted and thinned to 26 plants per plot, which gave a density of approximate 25,000 plants/acre. Planting dates were April 25 and May 12 for Environments 1 and 2, respectively. Fertilizer and herbicide were applied to the level of normal management practice in this area.

Flowering date for $F_{2: 3}$ lines was recorded as the number of days after June 30. Anthesis and silk emergence were recorded when $50 \%$ of the plants in a plot shed pollen or had exposed silks, respectively. Days after June 30 were transferred to the accumulated heating units (growing degree days, GDD) according to the following process (Shaw, 1988):
$\operatorname{GDD}\left(\mathrm{F}^{\circ}\right)=[($ daily maximum temperature+daily minimum temperature)/2]-50.
Maximum and minimum temperatures were $86^{\circ} \mathrm{F}$ and $50^{\circ} \mathrm{F}$, respectively. Five random plants from each plot were measured for plant stature. Plant height
and ear height were measured in centimeters from soil level to the top of the tassel and the node of the primary ear, respectively.

The following abbreviations were used in the Result and Discussion, PT for plant height, ET for ear height, ATS for anthesis measured in growing degree days (GDD) and ASL for silk emergence in GDD.

## Lab Analysis for RFLP Patterns

DNA was isolated from leaf tissue sample of each $F_{2: 3}$ family following the same procedure as described by Jarioe (1993). One hundred and thirteen maize genome and CDNA clones were selected based on map location and the pattern of polymorphism between the two parental lines for this population in the preliminary screening. The clones were from several sources, Brookhaven National Laboratory (BNL), University of Missouri, Columbia (UMC), Native Plants Incorporated (NPI), Pioneer Hi-Bred International, Inc. (PIO) and Iowa State University (ISU). The procedure for reatriction digestion and hybridization was described by Jarboe (1993).

## Statistical Analysis

Analysis for QTL location and population parameters were conducted on the basis of $F_{2: 3}$ progeny means. ATS and ASL were collected as progeny means. Five records each for PT and ET were averaged for the following analyses using the procedures described by Jarboe (1993).

The Shapiro-Wilk test was conducted for a test of normality among $\mathrm{F}_{2: 3}$ line means in SAS (SAS Institute, 1988). Parameters presented were mean, range, correlation coefficient and probability of the sample not derived from a population with a normal distribution.

Analysis of variance (ANOVA) in lattice design was conducted in PLABSTAT (Utz, 1972). Efficiency of blocking was calculated and traits with a relative efficiency larger than $105 \%$ were analyzed by adjusted means
in the mapping procedure and correlation study.
ANOVA for a random block design was conducted in SAS (SAS Institute) if the relative efficiency was less than $105 \%$ in lattice design.

Estimates for heritability were obtained on the basis of $F_{2: 3}$ progeny (Hanson, 1963) and confidence intervals were estimated following Knapp's (1985) procedure. Phenotypic correlations among traits were calculated according to Falconer (1989) definition. Computation was conducted in SAS (SAS Institute, 1988).

ANOVA in a lattice design showed the efficiencies of lattice design were 107.8\%, 121.4\%, $127.0 \%$ and $100.3 \%$ for ATS, ASL, PT and ET in Environment 1, 110.2\%, 105.9\%, 114.0\% and 150.5\% for the four traits in Environment 2. The adjusted means were used in QTL location procedures for all the traits except for ET in Environment 1 for which the efficiency was smaller than 105\%.

A linkage map was developed in a previous study in this population (Jarboe, 1993) using the software MAPMAKER (Lander and Botstein, 1989; Lincoln et al., 1990). This linkage map was used for QTL location in this study since all probes included were the same in the two studies. Two important parameters (threshold LOD score and recombination value) for construction of a linkage map were derived according to the principles elucidated by Lander and Botstein (1989). The LOD threshold and recombination values were 3.0 and 0.3 , respectively. One hundred and six probes were mapped to 10 linkage groups. The other seven probes were fit into the 10 groups by relaxing recombination value.

Putative QTL were identified in Environment 1 and 2 in the following two procedures. Linear regression model for single markers was analyzed to define the association of QTL with individual marker loci (Edwards et al., 1987). This analysis provided three tests (Appendix 9), for the total variation of the marker region contributing to the trait performance, each
for additive and dominance variation to the trait performance. Since 113 marker loci were included in the analysis, a large number of tests were conducted. A significant level of 0.001 was used on a per contrast basis to avoid increasing level of Type $I$ error. Single marker analysis allowed tests of significance for additive and dominance variation.

Interval mapping (Lander and Botstein, 1989) was used to test the possibility of $Q T L$ present in the interval defined by a pair of marker loci. The whole genome was scanned in the free genetic model. Individual regions with the highest probability of containing QTL were identified. The threshold LOD was 2.5 according to the procedure described by Lander and Botstein, 1989). No constraints were imposed because the free genetic model generated the results with highest likelihood and allowed multiple loci analysis. Multiple loci analysis (Lincoln et al., 1990b) was conducted in a additive model among loci. All regions identified in the whole genome scan were analyzed simultaneously for genetic effects. The most likely position of QTL in each interval was indicated. Estimates of additive effect indicated the direction of gene effects and the parent with high or low effects for the trait. Estimates of dominance effect indicated the performance of the heterozygous individuals.

The description of two methods indicated complementary information can be obtained from two analyses. When the probes were evenly distributed along the chromosome and had high density (less than 10 cM between pairs of markers), the results from two procedures should be the same. Interval mapping reached high precision by reducing the interference of double crossing-over. Single marker analysis might provide useful information in two ways: 1) When interval mapping identified a region flanked by two distant markers, single marker analysis might only detect the marker closer to the gene(s) for the trait and exclude a large region from the interval; and 2) Variation caused by additive and dominant effects could be tested.

Estimation of variation might contribute direct information to breeders. Interval mapping only estimated additive and dominant effect values. In general, first degree statistics (e.g. means) were not as tolerant to bias as second degree statistics (e. g. variance). Bias could be caused by recombination values and linkage disequilibrium. Additive and dominant variation could be tested and provide a significant test for the variation in the population.

Putative QTL were located for each environment and the results were compared. Chromosome regions were examined for highly correlated traits to investigate if $Q T L$ for correlated traits were detected on the same regions.

## Construction of Linkage Map

The linkage map was developed with 113 maize genomic and cDNA clones as described by Jarboe (1993). Total genetic distance covered was 1504.5 CM and the average distance between each pair of markers was 13.3 cm .

The average genomic composition of the $F_{2: 3}$ lines used for aingle row plots was 23.7\% homozygous for Mol7, 22.7\% homozygous for B52 and 53.6\% heterozygous (Figure 1). Overall, the composition exhibited a symmetrical pattern according to the means. This symmetry was expected for a sample from a normal distribution .

## Biometrical Analysis

On average, ATS and ASL had higher GDDs in Environment 2 than in Environment 1. Higher PT and ET were found in Environment 1 (Table 1). These results could be explained by the prevailing climatological conditions described in the two environments (see Material and Methods). In Environment 1, precipitation was higher than normal, and heat unit accumulation was similar to normal conditions. More heat units were accumulated in Environment 2 and precipitation was much below normal. Water stress and high rate of heat unit accumulation probably led to the shorter plant stature in Environment 2 than in Environment 1. Distribution of $F_{2: 3}$ Line Means

In Environment 1, $F_{2: 3}$ line means fit a normal distribution for PT and ET. ASL exhibited deviation ( $\alpha=0.01$ ) and ATS had severe deviation. Severe deviation from normality was detected for all traits except for PT in Environment 2. The deviation from normality of ASL and PT was probably due to one extreme high value of 1300 (GDD) for silk emergence date and several relatively high values for plant height (Table 1 and appendix 7). This
suggested deviation was not caused by the scale of the measurement because there was no skewed trend to certain direction(s). Transformation of original data was not performed. Variation Analysis for $\mathrm{F}_{3}$ Lines

Genetic variation was highly significant for all traits in both environments (Table 2). ATS and ASL had high heritability estimates (>80\%) in both environments and for combined data, while the heritability estimates were different for PT and ET in two environments. Estimates of heritability were much higher in Environment 1 (>75\%) than in Environment 2 (<45\%). A significant genotypic by environment interaction effect was detected for ATS only. Correlation Analysis

Phenotypic correlations between ATS and ASL were high for combined data combined in each environment ( $r \geq 0.7$ ). High phenotypic correlations between PT and ET were found in Environment 1 and combined data. Intermediate phenotypic correlations ( $0.3<r<0.7$ ) were found in Environment 2 for PT and ET (r=0.61) (Table 3). Correlations among other traits were in the intermediate range, except for the correlation between ATS and ET (r=0.16). All the correlation coefficients were highly significant $(\alpha=0.01)$, except for that of ATS and ET which were significant at the 0.05 level.

## QTL location and Analysis for the Regions Identified

 GDD to AnthesisThe whole genome scan indicated putative QTL for ATS were assigned to five regions, chromosomes 1, 2, 3, 5 and 8 in Environment 1 (Table 4 and Figure 2). The regions with largest effects were on chromosomes 1 and 8, as indicated by the highest LOD scores (4.9 for the region on chromosome 1 and 12.0 for the region on chromosome 8 ) and determination factor, $R^{2}$
(18.3\% for the region on chromosome 1 and $43 \%$ for that on chromosome 8). The determination factor indicated the proportion of total genetic variation explained by this region. Multiple loci analygis showed the length of the region on chromosome 1 was 17.7 cM and the most possible location of the QTL in the interval was 4.4 cM from NPI234. Estimates of additive and dominance effects were 18.8 and -47.2 GDD, respectively. The positive estimate of additive effect (18.8) indicated the alleles for earlier anthesis was from Mol7. The negative estimate of dominance effect (-47.2) suggested dominance would decrease the value of ATS. Dominance effects tended to promote early anthesis. Single marker analysis detected significant dominance variation for this region. The other three regions identified in Environment 1 had relatively smaller effects reflected by the lower LOD scores and $R^{2}$. The region on chromosome 2 had estimates of additive and dominance effects of 13.4 and 6.8 GDD , respectively; the region on chromosome 3 had 1.6 and -49.6 GDD, the region on chromosome 5 had 20.9 and -35.0 GDD, and the region on chromosome 8 had 47.5 and -30.0 GDD. Single marker analysis detected significant dominance variation in all regions except for the region on chromosome 3. Mo17 contributed alleles for earlier anthesis for all five regions. Three of the five regions were detected in Environment 2. Again, the region on chromosome 8 had the highest LOD (6.2) and explained a large amount of phenotypic variation (21.8\%). All regions identified in Environment 1 and two regions of largest effects identified in Environment 2 derived alleles for earlier anthesis from Mol7. Two regions detected in Environment 2 derived alleles for earlier anthesis from B 52 , including the region on chromosome 3 with additive and dominance effects estimated as 1.5 and -5.7 GDD, and the region on chromosome 10 with estimates of additive and dominance effects as -7.2 and -25.2 GDD. The region on chromosome 10 was only detected in Environment 2 by interval mapping. Single marker analysis did not detect
this region. The detection of this region was mostly due to the large distance between the two loci flanking the interval. Two region derived alleles for early anthesis from Mol7, including the region on chromosome 2 with estimates of additive and dominance effects as 17.7 and 45.8 GDD , and the region on chromosome 8 with estimates of additive and dominance effects as 28.2 and -3.4 GDD. In most cases, single marker analyses generated the same results as interval mapping regrading QTL location. Only in the large interval like the one on chromosome 10 , single marker analysis did not provide confirmation evidence for gTL existence. Dominance variation was the major source of variation for most regions except for chromosomes 3. The region on chromosome 8 had the largest effects. Substitution of B52 alleles with Mol7 alleles on this region would cause earlier anthesis by 47.5 GDD in Environment 1 and 28.2 GDD in Environment 2 in only this region was considered. Single marker analysis provided similar information as interval mapping for most regions. When QTL were identified near one of two flanking marker loci, the loci closer to QTL tended to have low probability values (NPI234 in Environment 1). When QTL were identified near the middle of the intervals by interval mapping, both marker loci tended to have significant probability values (NPI268-UMC89 in Environment 1 and 2). Regions with relatively low l\LOD score and large interval might not be detected by interval mapping (PIO20.0075-UMC64 in Environment 2). GDD to Silk Emergence

QTL for ASL were detected on chromosomes 1 and 8 in both environments (Table 4 and Figure 3). Two intervals on chromosome 1 were identified in Environment 2. Estimates of additive effects were 46.8 and -58.3 GDD in Environments 1 and 2, respectively. One was adjacent to the interval identified in Environment 1. Estimates of dominance effects were -77.6 and -29.4 GDD in Environments 1 and 2, respectively. The change of the additive effect estimates might be related to the drastic change of
environmental factors in the two seasons. The region on chromosome 8 had the highest LOD and $R^{2}$ value in Environment 1 and intermediate $L O D$ and $R^{2}$ in Environment 2 compared to other regions identified in this environment. Estimates of additive effects were 71.5 and 44.3 GDD in Environments 1 and 2, respectively. Estimates of dominance effects were -45.8 and -27.2 GDD in Environments 1 and 2, respectively. Regions identified only in Environment 1 were on chromosomes 2 and 5 with lower LOD scores and $R^{2}$ values than the regions on chromosome 1 and 8. Additive effects were estimates as 37.5 and 59.1 GDD for chromosomes 2 and 5, respectively. Estimates of dominance effects were 13.2 and -92.0 GDD. Chromosome 6 and 9 were identified only in Environment 2. Additive effects were estimated as 55.7 and 30.4 GDD for chromosomes 6 and 9 , respectively. Dominance effects were estimated as -87.8 and 7.6 GDD. Single marker analysis indicated dominance variation was more important than additive variation for QTL in all regions. The positive estimates of additive effects from multiple loci analysis indicated alleles for early silk emergence were derived from Mol7 for all regions except for one on chromosome 1,BNL8.29-BNL15.18. Single marker analysis provided information similar to interval mapping. When QTL were identified in relatively large intervals and were closer to one of the tow marker loci, the marker closer to QTL tended to have lower probability values in the test (UMC78 and UMC78 in Environment 1, and NPI234, NPI286 and PIO10.0005 in Environment 2). QTL identified near the middle of the interval tended to have significant probability values for both marker loci ((UMC157-BPI234 and NPI268-UMC89 in Environment 1, and BNL8.29-BNL15.18 in Environment 2).

## Plant Height

QTL for PT were identified on chromosomes 1, 3 and 6 in Environment 1 (Table 5 and Figure 4). The region on chromosome 1 had the largest LOD and explained $23 \%$ of the total phenotypic variation. Estimates of additive
and dominance effects were -9.3 and -2.6 cm , respectively. Substitution of B52 alleles on this region with Mol7 alleles would increase PT by 9.3 cm in Env. 2 and 11.3 cm in Environment 2. Single marker analysis indicated dominance was the main source of variation in all regions. Multiple loci analysis indicated the alleles for shorter plant stature were obtained from B52. Estimates of additive effects were -5.0 cm for the regions on chromosome 3 and 6. Estimates of dominance effects were 14.2 and 7.4 cm for regions on chromosomes 3 and 6, respectively. The same interval on chromosome 1 was identified for PT in Environment 2. BNL15.18-UMC128 exhibited both additive and dominant variation from single marker analysis. This region had a high LOD score of 8.7 and explained 25.9\% of the total phenotypic variation. Multiple loci analysis indicated alleles for shorter plant stature were derived from B52. Regions on chromosomes 3 and 6 identified in Environment 1 had relatively smaller effects with relatively low LOD scores ( 3.6 and 2.8 for chromosomes 3 and 6 , respectively). The phenotypic variation explained ( $\mathrm{R}^{2}=11.8$ for chromosome 3 and $R^{2}=8.2$ for chromosome 6) was lower than the region on chromosome 1 . Single marker analysis indicated both regions had exhibited dominance variation. Single marker analysis identified the marker locus to which QTL were close (BNL15.18 and Pll in Environment 1, and BNL15.18 in Environment 2). A large interval identified by interval mapping (PIO20.0075-UMC64) was not detected by single marker analysis.

## Ear Height

QTL for ET were identified in one region on chromosome 1 in Environment 1 (Table 5 and Figure 5). Additive effects were estimated as --11.3 and -13.6 cm in Environments 1 and 2 , respectively. Dominance effects were estimated as 2.4 and 1.8 cm . Single marker analysis indicated dominance was the main source of genetic variation. The alleles for lower ear were derived from B52. QTL for ET were identified on chromosome 1, 5,

7 and 8 in Environment 2 (Table 5). QTL on chromosome 1 had the highest LOD and explained $21.2 \%$ of the total phenotypic variation. B 52 alleles would cause 13.6 ET reduction on this region. QTL in BNLI5.18-UMC128, BNL8.33-NPI268 and near NPI268 had additive and dominance variation contributing to the genetic variation. QTL near UMC80 exhibited mainly additive variation. The most important region, BNL15.18-UMC128 and one of the minor regions, BNL5.624-NPI234, derived alleles for lower ear placement from B52. Estimates of additive effects were $-3.6,2.3$ and 3.2 cm for the regions on chromosomes 5, 7 and 8, respectively. Estimates of dominance effects were $-3.8,5.8$ and 2.8 cm . Single marker analysis identified similar regions as interval mapping. Marker loci closer to QTL tended to have lower probability values (BNL15.18 in Environment 1 and UMC8O in Environment 2).

## DISCUSSION

## Putative QTL Identified across Environments

In a related study, Jarboe (1993) located QTL for yield and yield components. QTL for the same traits were identified on the same chromosome regions for most of the cases when the region had relatively high LOD scores, while regions with relatively smaller effect tended to be detected on different chromosomes. Two possible explanations were given. First, the change of QTL location was genetically based. Different chromosome regions affected the trait or the same region functioned at different levels. Alternatively, the difference could be attributed to environmental effects. Similar results were obtained in this study for morphological traits. Stuber et al. (1992) evaluated the backcross populations from Mol7 and 873 in six diverse environments, little evidence for genotype by environment interaction was found. This result supported the hypothesis that regions with major effect(s) affected traits across environments. Paterson et al. (1991) reported 29 QTL for fruit traits in a tomato population. Four of the 29 regions were identified in three environments, 11 in two environments. No relationship between the effects of regions and identification over environments was discussed.

The climatic conditions in the two growing seasons provided environments in which the influence of one factor, precipitation, could be isolated and analyzed. As described in the Material and Methods section, Environment 1 had above average precipitation; however, Environment 2 had lower precipitation than average (Appendix 11). If the differences in QTL location was due to genetic factors only, the regions detected in two years would reflect the QTL under normal and water-stress conditions. QTL identified in two environments would function in normal and water-stress conditions. QTL identified in Environment 1 only functioned during the
normal precipitation conditions. QTL identified in Environment 2 only would function in water-stress condition.

Components of genetic variation varied among regions. For example, QTL for ATS exhibited more dominant variation on chromosome 8 , but more additive variation on chromosome 3. The regions detected in both environments seemed to have the same kind of genetic variation over environments. Regions on chromosome 8 for ATS and for ASL exhibited more dominant variation in two environments, the region on chromosome 3 had more important additive variation in both environments (Table 4).

In general, dominant variation was more prevalent. Two explanations were possible. 1) High dominance variation existed. Four traits studied were known to have high heterosis in hybrids. Dominance effect was important for these traits (Hallauer et al., 1988); 2) Overestimation of dominance variance existed. $F_{2: 3}$ lines were used in this study. Maximum linkage disequilibrium existed in $F_{2}$ population and one of the consequences was overestimation of dominance effects (Gardner et al., 1953; Gardner, 1963). Random mating of $F_{2}$ populations can break the linkage.

Among four morphological traits, significant genotype by environment interaction was only detected for ATS. From LOD score plots (Figure 2) and mapping results (Table 4), large discrepancies between the two environments for ATS on three chromosome regions. Regions on chromosomes 1 and 10 had QTL detected in only one environment. The region on chromosome 8 was identified in both environments, but at very different levels of effects. These three regions could be considered as component(s) of the genotype by environment interaction. Significant GxE interactions were not detected for ASL, PT and ET (Table 2), although relatively large discrepancies were observed in ASL and PT regarding LOD score and $R^{2}$ values (Table 4 and 5). Large difference of LOD scores was observed on chromosome 3 for PT (Figure 4). The difference in only one region with relatively small effect(s) was
not sufficient to be detected in the analysis of pooled effects of all genetic factors. This explanation could not provide a satisfactory answer for ASL. Chromosomes 1, 2 and 9 were identified in one of the two environments. Chromosome 1 included two regions showing larger difference between two environments (Figure 3). The most possible explanation would be the interaction in different directions among regions canceled each other in the analysis of pooled effects. Examining the four regions, one region each on chromosomes 1 and 2 were identified in Environment 1 and one region each on chromosomes 1 and 9 were identified in Environment 2. The directions of interaction of the four regions were different and would cancel the effects of each other. Therefore, RFLP could separate the effect(s) of different regions and be used to analyze individual chromosome regions for quantitative traits.

The region identified for ATS identified on chromosome 3 derived alleles for early anthesis from Mol7 in Environment 1 and from 852 in Environment 2. Two explanations were possible. Firstly, this region had LOD score of 2.6 in both environments. Estimates of the regions with relatively low effects were exposed to more bias because environmental factors played more important role(s) to the phenotypic values for regions with smaller effect(s). Especially the estimates were based on the first degree statistics (effects). Secondly, the climatological conditions were dramatically different in two environments, QTL might have different reaction to striking changes of environments.

## QTL Location in Different Studies

ATS, ASL, PT and ET were studied in the same population in a previous study in hill plots in difference environments (Jarboe, 1993). QTL for ATS identified on the same chromosomes included chromosomes 8 with major dominance variation. Chromosomes 1, 5 and 8 were identified containing QTL for ASL in both studies. Dominant variation was the main source of genetic
variation for both regions. QTL for PT identified in both studies included chromosomes 1, 5 and 6. Chromosomes 1 and 8 were identified for ET in both studies. QTL on chromosome 1 exhibited major dominant variation, while gTL on chromosome 8 had predominant additive variation.

From the current results, there was not an indication if the fluctuation of QTL detection over environments in the same population was due to 1) true genetic cause, i.e. different chromosome regions were involved in different environments for the same trait or 2 ) variation in the ability of the procedures to detect the $Q T L$ when the environmental factors varied.

Putative QTL for plant height were analyzed in different maize populations. Schön et al. (1993) reported QTL for PT on chromosomes 1, 3 and 9. One of the parents, $B 52$ used to create Schön's $F_{2}$ population was also used in the current study. Two of the three chromosomes identified by Schön et al., chromosomes 1 and 3, were detected in the B52 x Mol7 population with major effects. Chromosome 6 was detected in our study, not reported by Schön et al. The $p 1$ locus defining the region on chromosome 6 was not included in the linkage map used by Schön et al. This might be the cause chromosome 6 was not detected there. Beavis et al. (1991) reported 11 RFLP loci on eight of 10 chromosomes associated with QTL for PT in four $F_{2}$ maize populations. Different parents were used in each population. Sampling size for four populations varied from 112 to 144 and the number of genetic markers used for different populations varied from 68 to 148 . None of the 11 regions was detected in four populations. QTL on chromosomes 1, 3 and 9 were detected in two of the four populations. Others were only detected in one of the four populations. Their sampling size might be sufficient for a trait with a high heritability like PT. Change in number of marker loci might influence the ability to detect QTL in populations. All chromosomes detected in our study were identified in study by Beavis et
al.(1991).
Classical genetics studies had located at least 40 loci affecting PT (Coe et al., 1988). D8, a dwarf plant locus on chromosome 1, was in the vicinity of the interval BNL15.18-UMC128 identified for PT. The same region was detected in the hill plot results (Jarboe, 1993). The results suggest quantitative and qualitative loci for related traits distributed on the same chromosomes or in the nearby regions.

Two important considerations for accessing QTL are adequate number of markers and accuracy of trait evaluation over environments. Uniform and high density of probes over the genome is critical for studies using RFLP markers (Smith et al., 1991). Accurate measurement of traits permits the detection of QTL. Unless there was control of experimental error, the cause of the change of QTL locations over environments and populations could not be resolved. Increasing sample size and probably more sophisticated statistical modeling and designs are needed to make QTL location useful to plant breeders.

Table 1. Summary Statistics and Normality Test for Morphological Traits on the Basis of $F_{2,3}$ Line Means


[^1]Table 2. Variation Analysis and Heritability Estimates for Morphological Traits

| Trait | Env. | $\mathrm{h}^{2}$ |  | $\sigma_{8}^{2}$ | $\sigma_{p e}{ }^{2}$ | $\sigma_{e}^{2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| ATS | 1 | 81.7 | (74.9-86.7) | 2080.4** |  | 930.7 |
|  | combined | 86.7 | $(81.7-90.3)$ |  |  |  |
|  |  | $83.7$ | $(77.7-88.1)$ | $1636.8$ | 291.7** | $689.3$ |
| ASL | 1 | 88.5 | (84.2-91.6) | 8272.8************) |  | 2149.3 |
|  | $2$ | 84.5 | (78.7-88.7) | $6696.8$ |  | 2458.4 |
|  | Combined | 93.1 | (90.8-95.1) | $7530.9 * *$ | 0 | 2252.0 |
| PT | 1 | 83.3 | (77.0-87.8) |  |  | 70.0 |
|  | 2 | 42.8 | (25.4-56.1) | $99.0$ |  | 265.1 |
|  | Combined | 70.5 | (61.5-77.3) | $119.9 *$ | 17.0 | 167.0 |
| ET | 1 | 78.0 | (69.9-84.0) |  |  | 38.7 |
|  | 2 | 40.1 | (22.6-54.4) | 58.7************) |  | 192.1 |
|  | Combined | 65.9 | (53.2-75.2) | $57.3 *$ | 9.5 | 99.4 |

[^2]Table 3. Phenotypic Correlation Coefficients among Traits on the Basis of $F_{2: 3}$ Iine Means ${ }^{\wedge}$

|  | ASL | PT | ET |
| :---: | :---: | :---: | :---: |
| Combined Data |  |  |  |
| ATS | $\begin{gathered} 0.79 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.48 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.36 \\ (0.0001) \end{gathered}$ |
| ASL |  | $\begin{gathered} 0.49 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.16 \\ (0.0427) \end{gathered}$ |
| PT |  |  | $\begin{gathered} 0.72 \\ (0.0001) \end{gathered}$ |
| Env. 1 |  |  |  |
| ATS | $\begin{gathered} 0.79 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.41 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.39 \\ (0.0001) \end{gathered}$ |
| ASL |  | $\begin{gathered} 0.35 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.33 \\ (0.0001) \end{gathered}$ |
| PT |  |  | $\begin{gathered} 0.73 \\ (0.0001) \end{gathered}$ |
| Env. 2 |  |  |  |
| ATS | $\begin{gathered} 0.76 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.33 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.42 \\ (0.0001) \end{gathered}$ |
| ASL |  | $\begin{gathered} 0.50 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.30 \\ (0.0001) \end{gathered}$ |
| PT |  | (0.0001) | $\begin{aligned} & 0.61 \\ & (0.0001) \end{aligned}$ |

A numbers in the bracket are probability values for test of significance for correlation coefficients

Table 4. Regions for GDD to Anthesis and Silk Emergence Identified by Interval Mapping

${ }^{\text {A Results from significance test by single marker analysis, at the significance level of } \alpha=0.001 \text {, } A, ~}$ indicated significant additive variation, $D$ indicated significant dominant variation, A/D indicated significant additive and dominant variation while additive variation had lower probability than dominance variation, $D / A$ indicated significant additive and dominant variation while dominance variation had lower probability than dominance variation, and - indicated no significant additive and dominance variation was detected.
${ }^{B}$ LP indicated the parent contributing alleles conferring lower value for the trait
c Position of QTL relative to the loci defining the interval, denoted by the distance from the loci on the left in the interval
D Determination factor from multiple loci analysis.
${ }^{\mathrm{E}}$ LOD score from multiple loci analysis.
F Estimates of gene effects from multiple loci analysis. Add. and Dom. indicated additive and dominance effects, respectively.

Table 5. Regions for Plant and Ear Height Identified by Interval Mapping

| Chr. | Interval | Summary Scan |  | Multiple Loci Analysis |  |  |  | GAA | $L P^{8}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | peak | $\mathrm{R}^{2}$ (\%) | Length | Position ${ }^{\text {c }}$ | Gene | ects |  |  |
|  |  | LOD |  | (cM) | (CM) | Add. | Dom. ${ }^{\text {F }}$ |  |  |
| Plant Height, Env. 1 |  |  |  |  |  |  |  |  |  |
| 1 | BNL15.18-UMC128 | 5.9 | 23.0 | 25.4 | 8.2 | - 9.3 | - 2.6 | D | B52 |
| 3 | UMC60- UMC165 | 3.6 | 11.8 | 10.3 | 9.3 | - 5.0 | 14.2 | D | B52 |
| 6 | P11-UMC85 | 2.8 | 8.2 | 26.7 | 7.5 | - 5.0 | 7.4 | D | B52 |
|  | Sum | 12.3 | 43.0 | $\mathbf{R}^{2}=4$ | 0.1\% ${ }^{\text {D }}$ | LOD=12 |  |  |  |
| Plant Height, Env. 2 |  |  |  |  |  |  |  |  |  |
| 1 | BNL15.18-UMC128 | 8.7 | 25.9 | 25.4 | 8.0 | -11.3 | 2.4 | D/A | B52 |
| Eax Height, Env. 1 |  |  |  |  |  |  |  |  |  |
| Ear Height, Env. 2 |  |  |  |  |  |  |  |  |  |
| 1 | BNL15.18-UMC128 | 5.6 | 21.2 | 25.4 | 7.6 | - 5.3 | - 1.2 | D | B52 |
| 5 | BNL5.624-ENL6. 25 | 2.7 | 9.4 | 14.9 | 7.1 | - 3.6 | - 3.8 | D/A | B52 |
| 7 | UMC80- BNL8. 39 | 2.7 | 8.2 | 21.4 | 0.5 | 2.3 | 5.8 | D/A | Mo17 |
| 8 | NPI268-UMC89 | 2.8 | 10.2 | 25.5 | 8.7 | 3.2 | 2.8 | D/A | Mo17 |
|  | Sum | 13.8 | 49.0 | $\mathrm{R}^{2}=4$ | 1.0\% ${ }^{\text {D }}$ | LOD=13. |  |  |  |

${ }^{\text {A }}$ Results from significance test by single marker analysis, at the significance level of $\alpha=0.001, A$ indicated significant additive variation, $D$ indicated significant dominant variation, A/D indicated significant additive and dominant variation while additive variation had lower probability than dominance variation, $D / A$ indicated significant additive and dominant variation while dominance variation had lower probability than dominance variation, and - indicated no significant additive and dominance variation was detected.
${ }^{1}$ LP indicated the parent contributing alleles conferring lower value for the trait
c position of gTL relative to the loci defining the interval, denoted by the distance from the loci on the left in the interval
D Determination factor from multiple loci analysis.
E LOD score from multiple loci analysis.
F Estimates of gene effects from multiple loci analysis. Add. and Dom. indicated additive and dominance effects, respectively.


Figure 1. Genomic Composition for Population $852 \times$ Moit. $X$ axis indicated the percentage of $B 52$, Mo17 and B52 x Mo17 components. Y axis indicated the number of individuals.

C1


C2


C3


C4


C5


Figure 2. Plot of LOD Score for Anthesis (GDD) Detected for Each Environment.
The $X$ axis represents the chromosome with vertical bars indicating the distribution of RFLP loci. The $Y$ axis is the scale of LOD scores.

C6
C7


C8


C9



C1


C2


C3


C4


C5


Figure 3. Plot of LOD Score for Silk Emergence date (GDD) Detected for Each Environment.
The $X$ axis represents the chromosome with vertical bars indicating the distribution of RFLP loci. The $Y$ axis is the scale of LOD scores.


C7


C8




Env. 1
Env. 3

C1


C2


C3


C4


Figure 4. Plot of LOD Score for plant Height Detected for Each Environment.
The $X$ axis represents the chromosome with vertical bars indicating the distribution of RFLP loci. The $Y$ axis is the scale of LOD scores.

## C5 <br> 

C6


C7


C8



Env. 1
_ Env. 3

C1


C2


C3


C4


C5


Figure 5. Plot of Lod Score for Ear Height Detected for Each Environment.
The $X$ axis represents the chromosome with vertical bars indicating the distribution of RFLP loci. The $Y$ axis is the scale of LOD scores.

C6



C8




Env. 1

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# PAPER III. LOCATION AND ANALYSIS OF CHROMOSOME REGIONS FOR GRAIN YIELD COMPONENT TRAITS IN A <br> maize population 

## ABSTRACT

Grain yield is the primary trait in most maize improvement programs. Several plant, ear and kernel traits have been considered as the components of grain yield. In this study, RFLP markers were used to detect and analyze chromosome regions conferring grain yield and seven yield component traits. The linkage map was developed by 113 maize genomic or cDNA clones. Replicated evaluation of $150 \mathrm{~F}_{2: 3}$ lines was conducted in two environments, 1990 and 1991, at Ames Agronomy Research Farm. Conditions in 1990 reflected above average precipitation and normal heat unit accumulation. Water stress and a high rate of heat unit accumulation were observed in 1991.

Location and effects of QTL varied between environments. Overall, the regions with larger effects were detected in both environments and regions with smaller effects tended to be detected in only one environment.

Because of the quantitative inheritance pattern of most agronomic traits, evaluation over environments is essential. The Marker by Environment component was partitioned in a linear model to quantify the marker-QTL association across environments. The analysis was able to discriminate regions which contributed differently to the trait performance in two environments. This method of partitioning the variation component due to Marker by Environment interaction provided a potential new way to analyze marker data across environments.

## INTRODUCTION

Analysis of quantitative trait loci ( $Q T L$ ) was not possible until the development of molecular markers. Restriction Fragment Length Polymorphisms (RFLPs), have shown great potential for dissecting QTL for traits of economic value in breeding programs (Tanksley et al., 1989).

Putative QTL for traits related to grain yield have been detected in maize. Stuber and Edwards (1987) located QTL for 24 yield component traits by isozyme markers and concluded molecular markers were effective for identifying QTL in maize populations. Similar results were reported by Abler et al. (1991) in populations resembling those used in breeding programs. Stuber et al. (1992) reported results from studies related to heterosis and genotype by environment interaction. QTL for grain yield were mapped to nine of the 10 chromosomes. When $Q T L$ for grain yield were detected, heterozygous genotypes had higher phenotypic values than the respective homozygous classes, suggesting a relationship between QTL for yield and expression of heterosis. Little evidence for genotype by environment interaction was detected (Stuber et al., 1992).

QTL for agronomic traits were located for other crop species. Paterson et al. (1988, 1991) located QTL for fruit traits in an interspecific Lycopericon population. One of the studies by Paterson et al. (1991) was conducted across three environments. Total of 29 regions were detected; however, only four of the 29 were identified in three environments. Eleven regions were identified in two environments and others were only detected in one environment. The difference of QTL location for the same traits in different environments was reported by Schön et al. (1993). The consistency of QTL location is a concern for breeders, as it is directly related to the utility of the results from marker-facilitated studies in breeding programs.

Grain yield is commonly the top priority of maize breeding programs (Hallauer et al., 1988). In the past 50 years, U.S. and world corn production has experienced dramatic changes which led to the significant grain yield increase (Duvick, 1977; Russell, 1974, 1986). The results indicated genetic improvement has been made during the process of hybrid development. The genetic improvement contributed about $50 \%$ of the total grain yield increase in U.S. (Fehr, 1980). The other 50\% increase has been due to non-genetic factors such as chemical usage and improvement of cultivation practices. Knowledge of the genetic basis of grain yield and related traits will contribute to genetic improvement.

Grain yield is a complex trait (Hallauer and Miranda, 1988). Several traits have been found related to grain yield, such as plant stature, lodging resistance, pest and disease resistance and ear and kernel traits. Although selection directly on grain yield has been more effective than selection of component traits, breeders have studied the potential of using correlated traits to maximize genetic gain in breeding programs (Hallauer et al., 1988). Genetic studies on the basis of individual loci may define the mechanism(s) behind correlations among traits and contribute to the genetic improvement of yield through correlated genetic gain.

Genotype by environment interaction is the difference of genotypes across environments. Two types of interaction exist. One type interaction is the change of magnitudes of genotypes in different environment. The other type is the change of the order of different genotypes across environments. Interaction between genotype and environment plays an important role in the inheritance of grain yield since a large number of loci control expression of this trait. The difference of the performance Of a genotype in various environments has complicates selection procedures because superior genotypes can not be identified in one environment. Evaluation across environments is necessary to obtain repeatable ranking of
genotypes. Evaluation across environments leads to the discussion of testing environments. One point of view is teating environments should be the same as the target area of plant breeders. The other point of view is testing should be conducted in nonlimiting conditions to allow the expression of all genes. Rosielle and Hamblin (1981) evaluated yield and tolerance to stress in stress and nonstress conditions. The conclusion from this study was that the most desirable approach would be to choose testing aites to be representative of population of environments for which breeders want to improve mean yield. Johnson and Geadelmann (1989) conducted recurrent selection for grain yield in a maize population AS-A under irrigated and dryland conditions. The results indicated selection for grain yield under irrigation gave superior results to those obtained from selection under dryland conditions. Selection under irrigation was as effective as selection under dryland conditions for increasing yield in moisture-stress environments.

In studies facilitated with molecular markers, scientists are concerned with following questions: 1) Are major QTL identified consistently on the same chromosome regions for the same traits? Will the results from markers reduce the scale of conventional breeding to a large extent? and 2) Are QTL detected on different chromosome regions across environment?

In this study, one objective was to identify the chromosome regions for eight components of grain yield, including ear number per plot, grain yield/plot, 300-kernel weight, number of kernel rows/ear, ear length, ear diameter, cob diameter and kernel depth. Another objective was to analyze marker $x$ environment interaction, and investigate if this partitioning would provide additional information for QTL identification over environments.

## MATERIAL AND METHODS

## Population Development

The segregating $F_{2}$ population was created by crossing inbred lines B52 and Mol7, and self-pollinating the $F_{1}$ hybrid. One hundred and fifty unselected $F_{2}$ plants were self-pollinated to produce $F_{2: 3}$ families. Fifteen kernels from each $F_{2: 3}$ family were planted in the greenhouse. An equal amount of leaf tissue was harvested from each of the 15 plants for each family for RFLP analysis. Sib-mating of the $F_{2: 3}$ lines was conducted to increase seed supply for field evaluation.

## Experimental Design

A $12 \times 13$ rectangular lattice design including $150 \quad \mathrm{~F}_{2: 3}$ lines and six bulked $F_{3}$ entries were planted in two replications in each of the two environments:

was 9.57 inches compared to the normal of 12.45 inches. Including September, the precipitation was 11.93 inches in 1991 and 15.57 inches for normal years. Accompanied by the drought condition, the accumulation of heat units (GDD) was more dramatic from May to September in 1991 (Appendix 11). By September, 3218 GDD heat unit were accumulated in 1991, about 270 GDD higher than normal condition (2941 GDD) (Carlson and Lamkey, 1992, personal communication).

Plots consisted of a single row of 18 feet long with 2.5 feet between adjacent rows. plots were machine-planted and thinned for 26 plants per plot to a final density of 25,000 plant/acre. Planting dates were April 25 and May 12 for Env. 1 and 2, respectively. Fertilizer and herbicide were applied during spring and early summer at the level of common management practice in this area.

Morphological traits (anthesis, silk emergence, plant height and ear height) were measured before harvest. Plots were hand harvested and dried to a uniform moisture. The primary ear and secondary ear were kept separate in the process of data collection. Data were taken on number of primary ears per plot (ears/plot) (EN). Ten random primary ears from each plot were measured for the following traits: ear length (cm) (EL), ear diameter ( Cm ) (EW) and number of kernel rows per ear (rows/ear) (KR). The average was used for final analysis. All ears were then shelled and grain yield per plot (GY) was recorded as grams per plot. A sample of the shelled grain in each plot was saved to permit a measurement of 300 -kernel weight ( $g$ ) ( KW ). Ten cobs from each plot were used to record the average cod diameter (cm) (CW). Kernel depth (cm) (KD) was derived by subtracting average cob diameter from the ear diameter. Stand count was taken before harvest, and uniform stand was obtained (Appendix 6). No significant variation was detected for stand count in either season. Grain yield/plot was analyzed without covariance adjustment for stand count (Cochran and

Cox, 1957).
DNA for $F_{2: 3}$ families was obtained for RFLP analysis as described by Jarboe (1993). One hundred and thirteen maize genomic and CDNA clones were selected to construct the linkage map and identify the QTL. The clones were derived from Brookhaven National Laboratory (BNL), University of Missouri, Columbia (UMC), National Planta Incorporate (NPI), Pioneer HiBred International, Inc. (PIO) and Iowa State University (ISU).

## Statistical Analysis

The following analyses and tests were conducted for field data using the procedures described by Jarboe (1993).

1. Shapiro-Wilk test was conducted to test the normality among $F_{2: 3}$ line means in SAS (SAS Institute, 1988).
2. ANOVA in lattice deaign was conducted in PLABSTAT (Utz, 1987) to analyze the variation in each experiment.
3. ANOVA in RBD was conducted if the relative efficiency was less than 105\% in lattice design (SAS Institute, 1988). Lattice design had an efficiency of $101.8 \%$, 103.5\%, 100.0\%, 100.1\%, $100.0 \%$ and $100.5 \%$ for ear number, grain yield, 300 kernel weight, kernel rows, ear length, ear width, cob width and kernel depth, respectively in Env. 1, and 100.4\%, 105.7\%, 101.2\%, 100.0\%, 101.3\%, 100.4\%, 100.0\% and $100.0 \%$ in Env. 2. All traits were analyzed by unadjusted means except for grain yield in Environment 2.
4. Estimates for heritability were obtained on the basis of $F_{2: 3}$ line means (Hanson, 1963). The confidence intervals for heritability estimates were calculated according to Knapp et al. (1985). Phenotypic correlations were calculated (Falconer, 1989).

The linkage map was developed in MAPMAKER 2.0 (Lincoln et al., 1990a) with parameters of LOD threshold 3.5 and recombination value of 0.3. One hundred and six probes were included in the first step. The remaining
seven probes were fit into 10 linkage groups by relaxing the recombination value.

Putative QTL were identified in Env. 1 and 2 by a linear regression model for single markers (Edwards et al., 1987) and interval mapping (Lander and Botstein, 1989). In the linear regression analysis, a significant regression mean square of phenotype values on the marker classes indicated possible linkage relationship between QTL for the trait and the RFLP marker. Based on the number of markers included, i. e. the number of tests-conducted, the significance level for individual test was 0.001 following the description of Jarboe (1993). Interval mapping defined a maximum likelihood function to calculate the probability of QTL existing in the interval between a pair of marker loci. Jarboe (1993) described the derivation of a proper LOD threshold for this study following the procedure described by Lander and Botstein (1989). The LOD used in this study was 2.5. The chromosome regions with putative $Q T L$ in two environments were compared. Marker loci identified in isterval mapping were analyzed simultaneously in the multiple loci analysis (Lincoln et al., 1990b). The relative positions of QTL in the intervals were identified and additive and dominance gene effects were derived. The signs of the effects provided a means of determining the direction of the gene action and the direction of gene effect from each parental line. If the intervals detected covered a long region (interval>20-25 cM), results from single marker analysis might be able to exclude one of the two markers identifying the interval and locate QTL closer to one of the two marker loci involved.

A linear model was used to partition the marker $x$ environment interaction:

$$
\begin{equation*}
Y=E+L+(E x L) \tag{1}
\end{equation*}
$$

where $Y$ was the mean of $F_{3}$ lines over environments; E was the environmental effect;

L was the $F_{3}$ line effect which can be partitioned into two components of Marker (M) and Line/Marker (L/M);

ExL was the interaction between environment and $F_{3}$ lines
which can be partitioned into two components of
Environment $x$ Marker interaction (ExM) and
Environment x Line/Marker (ExL/M).
Then equation (1) can be written as
$Y=E+M+L / M+(E x M)+(E x L / M)$
The ExM component can be tested against ExL/M term and provided a test for interaction between marker and environments. The analysis was conducted in SAS (SAS Institute, 1988).

Since the markers tested for each trait for Marker x Environment interaction were tested at the same time, the significant level was $\alpha / n_{m}$ on a per contrast basis, where $\alpha$ was the overall significance level and $n_{m}$ was the number of markers involved in the test for each trait.

The probability values for the two marker loci identifying the interval were compared according to the relative position of QTL within the interval by multiple loci analysis. The purpose was to observe if the loci closer to QTL contributed more variation for interaction than the loci further from QTL when significant interaction was found.

## Linkage Map

Linkage map for population Mo17 x B52 was developed as described by Jarboe (1993). One hundred and thirteen genomic and CDNA probes identified loci covering 1504.5 cm of the genome with 13.3 cm between each pair of loci on average.

## Biometrical Analysis for Ear and Grain Traits

The contrasting environments for this experiment had obvious effects On GY and EN (Table 1). Water stress sondition(s) caused low EN (16 ears/plot) in 1991 compared to that of normal (18 ears/plot) in 1990. GY was 1.34 kg in 1990 under normal precipitation and heat unit accumulation. Only 1.18kg GY was achieved in 1991 under the drought condition. Similar effects of climatological conditions were found in another study on morphological traits(Jarboe, 1993). In 1990, the average PT was 246 cm vs. 209 cm in 1991. ET had an average of 97 cm in 1990 compared to the average of 82 cm in 1991.

## Distribution of $\mathrm{F}_{2: 3}$ Line Means

$F_{2: 3}$ line means over two environments fit normal distribution for five of the eight traits, GY, KW, EL, EW and CW. Three other traits showed severe deviation from normality, EN, EW and KD (Table 1 and Appendix 7). Most plants bore only one productive ear. Few plots (<5) had secondary ears and these ears did not produce very much grain. Therefore, the primary ear was included in the analysis. Several lines had barren ears and had low count for EN (Appendix 6). There were not any missing plots and stand counts were uniformly high in both growing seasons. Most of the stand count values were clustered near the highest number of 26 plants/plot (Appendix 7). Two reasons might cause the deviation from normal
distribution of $K D: 1$ ) one of the components in the calculation (EW) had a high degree of deviation; 2) the current measurement was not precise enough to classify the group of genotypes according to units of measure, especially when a linear combination (subtraction of two variables) was used to calculate values for the trait. Variation Analysig

Genetic variation was highly significant for all traits (Table 2). Heritability estimates were relatively high for EN, GY, KW, KR, EL and CW (66-75\%). Heritability estimates were low for EW (35\%) and very low for KD (7.9\%). The low heritability estimates of the two traits might be caused by the lack of accuracy of the measurements, especially for KD.

## Correlation Analysig

Among the 28 (among eight traits) pairs of correlation coefficients for $F_{2: 3}$ means over environments, only one (between $E N$ and $G Y$ ) reached a high correlation in both environments. Most (16 pairs) had intermediate correlations ( $0.3<r<0.7$ ). Eleven pairs had low correlation, and eight of the eleven were not significant ( $\alpha=0.05$ ). All correlation coefficients larger than intermediate were highly significant ( $\alpha=0.01$ ). The correlation between EN and GY (r=0.85 for Env. 1, r=0.89 for Env. 2), and between EW and KD (r=0.76 for Env. 1, r=0.87 for Env. 2) were high in both environments. Correlations between GY and $K D(r=0.75)$, and between $G Y$ and KD (r=0.82) were high in Env. 2. Correlations among other traits were intermediate or low in both environments.

Correlation varied between two environments for several traits (Table 3B, 3C). Some had significant or highly significant correlation coefficients, but in opposite directions in two environments, including the following pairs: EN and EW ( $r=-0.26^{* *}$ in Env. $1, r=0.61^{* *}$ in Env. 2), GY and CW ( $r=-0.16^{*}$ in Env. 1, $r=0.21^{* *}$ in Env. 2). More traits had significant correlation in only one of the two environments, including the following
pairs: GY and KR ( $r=0.07$ in Env. 1, $r=0.23^{* \prime \prime}$ in Env. 2), GY and EW ( $r=-0.01$ in Env. 1, $r=0.7^{*=}$ in Env. 2), EL and CW ( $r=0.08$ in Env. 1 and $r=0.44^{-3}$ in Env. 2), EN and KD ( $r=-0.11$ in Env. 1, $r=0.68^{* \prime \prime}$ in Env. 2), and GY and KD ( $x=0.04$ in Env. 1, $r=0.75$ in Env. 2).

Location of gTL for Ear and Grain Traiśs and Analysis of Regions Identified Grain Yield

Putative QTL for GY were identified on chromosomes 1, 3 and 5 in both environments. Also, QTL for GY were detected on chromosome 6 in Environment 2 by interval mapping (Table 4 and Figure 1). Estimates for additive effects were $-194.6,-18.2$ and -147.0 g for regions on chromosomes 1, 3 and 5, respectively, in Environment 1, and 280.0, -177.4 and -292.2 g in Environment 2. Estimates of dominance effects were 123.4, 348.6 and 419.6 g in Environment 1 and $473.8,-37.6$ and 403.0 g in Environment 2. The regions on chromosomes 1 and 5 had high LOD scores in both environments (LOD>4.0) and explained a high percentage of total phenotypic variation ( $R^{2}>25 \%$ ). The region on chromosome 3 did not have as high a LOD; however, the interval was much smaller than the other two. Multiple loci analysis indicated the most likely location and genetic effects of QTL in the interval. For example, QTL on chromosome 1 identified in Environment 1 were mostly located 16.2 cM from UMC67. The negative signs of additive effects for most regions (-194.6 g for the region on chromosome 1 identified in Environment 1) indicated genetic factors for low yield were derived from B52. The positive signs of dominance effects for most regions (123.4 for the region identified in Environment 1) indicated the dominant effect increased the performance of yield. This is a region with major effects because the LoD score was relatively high (4.1) and explained relatively high percentage of total phenotypic variation (17.1\%). Single marker analysis detected dominance variation in most of the regions except
for the QTL on chromosome 3 in Environment 1 and on chromosome 1 in Environment 2. One region on chromosome 6 was identified in Environment 2 with a relatively low LOD score of 2.6. Chromosome 1 was identified in both environments; however, large distances ( $>50 \mathrm{cM}$ ) existed between the regions identified in two environments. The large distance made it unlikely that QTL were detected on the game regions for the trait. In environment 1 , single marker analysis detected significant dominant variation. In Environment 2, additive variation was detected. The difference in the source of genetic variation might be the evidence that QTL detected on the same chromosome were in two independent regions. Mol7 contributed genetic factors for higher GY in Environment 1. In Environment 2, regions on chromosome 3 and 5 derived alleles for higher grain yield from Mol7. The other two regions on chromosomes 1 and 6 derived alleles for higher grain yield from B52. These result might indicate B 52 had relatively high perforce in water stress condition.

The climatological conditions in the two growing seasons provided contrasting environments with above average precipitation and normal accumulation of heat units in 1990 and water-stress accompanied by high rate of heat unit accumulation in 1991. Grain yield performance reflected the effects of the environmental conditions. The average $G Y$ of all $F_{2: 3}$ lines was 1338 (g) in Environment 1 and 1181 ( $g$ ) in Environment 2. If the differences in QTL locations were due to genetic factor(s), i.e. different chromosome regions, the difference in the mapping results might have revealed genetic factors that respond to contrasting environments. The other cause of variation could be due to the change of experimental condition only. As the variation components would vary in different environments, the tests conducted on the basis of these components would have different sensitivities. In one condition, a trait might not exhibit as large amount of variation as in other conditions. The same amount of
variation for a trait might have different amounts for components. Ear Number/plot

The regions with the highest LOD scores for EN (LOD>4.9) were identified on chromosomes 1 and 5 in two environments (Table 5 and Figure 2). Estimates of additive and dominance effects were -2.5 and 3.4 for chromosome 1 in Environment 1, 3.2 and 2.0 in Environment 2. For the region on chromosome 5 , additive effects were estimated as -2.0 and -2.9 in Environments 1 and 2, respectively. Estimates of dominance effects were 2.0 and 2.6. Single marker analysis indicated dominance variation was important for these regions. Genetic factors resulting in fewer ears were derived from $B 52$ in the two regions detected in Environment 1 and the region on chromosome 5 in Environment 2. Mol7 contributed alleles for fewer ears for the region on chromosome 1 in Environment 2. Two additional regions on chromosomes 3 and 8 were identified in Environment 1. Estimates of additive effects were 0.3 and -2.6 for the regions on chromosomes 3 and 8, respectively. Dominance effects were 3.0 and 2.6 . In these cases, genetic factors for fewer ears were derived from B52. In Environment 2, Mol7 contributed factors for fewer ears to the regions on chromosomes 1 and 6. Estimates of additive and dominance effects for the region on chromosome 6 were 2.3 and 1.6 , respectively. The additive effect of 2.9 indicated Mol7 alleles on this region on chromosome 5 would cause increase of two ears/plot in only this region was considered. Dominance effects tended to increase the ear number for all regions in both environments. Overdominance existed for the region on chromosome 1 in Environment 1 (3.4>2.5). Collectively, four regions identified in Environment 1 could account for $65.6 \%$ of the total phenotypic variation. The phenotypic variation explained by the three regions in Environment 2 was 49.5\%. Single marker analysis indicated more significant dominance variation for all regions detected in both environments except for the region on
chromosome 3 in Environment 1.

## 300-kernel Weight

QTL for KW were detected on chromosomes 1, 2 and 6 in Environment 1 (Table 6). Estimates of additive effects were -7.2, 3.1 and 3.1 for regions on chromosomes 1, 2 and 6, respectively. Estimates of dominance effects were $0.1,-5.0$ and -4.2 The region on chromosome 1 contributed both additive and dominant variation. QTL on chromosomes 2 and 6 seemed to contribute more dominant variation. Genetic factors for lower KW were derived from $B 52$ for the region on chromosome 1 and from Mol7 for the regions on chromosomes 2 and 6. Dominance effects reduced KW for the regions on chromosomes 2 and 6, and slightly increased KW for the region on chromosome 1. The region on chromosome 1 played an important role in the inheritance of the trait, explaining $31.3 \%$ of the total phenotypic variation. For individuals with $B 52$ component for this region, KW would be increased 7.2 g if this region was substituted with Mol7 component. However, dominance effect had very little contribution to heterosis expression ( 0.14 ). The three regions accounted for $48.3 \%$ of the total phenotypic variation.

QTL for KW were identified on chromosomes 2, 3 and 6 in Environment 2 (Table 6). Estimates of additive effects were 2.9, -3.1 and -2.3 for the regions on chromosomes 2, 3 and 6, respectively. Estimates of dominance effects were -2.2, 1.4 and 1.4. Single marker analysis showed significant dominance variation for all regions. Two regions on chromosomes 3 and 6 derived alleles for low KW from B52. The region on chromosome 2 derived alleles for low KW from Mo17. The total phenotypic variation explained by the regions (27.4\%) was much lower than that in Environment 1. Number of Kernel Rows/ear

QTL for KR were identified on chromosomes 1 and 5 in both environments (Table 7 and Figure 4). Estimates of additive effects were 0.39 and 0.5
for the region on chromosome 1 in Environments 1 and 2, respectively. Dominance estimates were 0.52 and 0.4 . Additive effects were estimated as 0.70 and 0.6 for chromosome 5 and dominance effects were 0.42 and 0.8 . Two intervals were identified on chromosome 1 in Environment 1. UMC128-UMC23 was the major contributor (LOD=3.4 for the interval of 8.6 cm ). The same interval was identified in Environment 2. Single marker analysis indicated additive variation was more important in Environment 1, but dominance variation was the major source of genetic variation in Environment 2. QTL identified on chromosome 5 had the largest effects on the inheritance of the trait, explaining more than $20 \%$ of the total phenotypic variation in both environments. Substitution of B 52 alleles on this region with Mol7 alleles would cause reduction of $0.6-0.7$ row/ear on average if only this region was considered. Additive variation was the more important source of genetic variation. One interval was detected on chromosome 9 in Environment 1 with more additive variation. Chromosome 6 was identified containing QTL for $K R$ in Environment 2 with more dominance variation. Estimates of additive and dominance effects were 0.4 and 0.02 . Mol7 contributed alleles for fewer kernel rows for all regions identified. Ear Lenath

QTL for EL were detected only on chromosome 3 in both environments. In Environment 1, three intervals appeared important for EL (Table 8). However, the three regions might not represent independent QTL for the trait. Interval UMC175-UMC50 was the unly interval detected by single marker analysis with significant dominant variation. The highest LOD was obtained for this region. Even through the total variation explained by the region ( $R^{2}=11.4$ ) was not as high as that of interval NPI457-UMC16, the interval covered a much smaller region ( 6.5 cm ) than NPI457-UMC16. The detection of the two other intervals was likely due to linkage and the larger intervals where the QTL were identified. B52 contributed genetic
factors for shorter ears in both environments. QTL identified on chromosome 3 in Environment 2 had the highest LOD and the largest effects, explaining 14\% of the total phenotypic variation. Three regions on chromosomes 1, 5 and 6 were only detected in Environment 2. However, the LOD score plots indicated chromosomes 1,5 and 6 were very close to reach significant LOD scores in Environment 1. Regions with more additive variation seemed to derive alleles for shorter ears from Mol7, whereas regions with more dominance variation derived alleles for shorter ears from B52. Dominance effects caused increased ear length for all regions. B52 contributed alleles for shorter ears for the regions with large effects on chromosome 3. The region on chromosome 3 denoted by UMC175 had the largest effect in both environments. Substitution of $B 52$ alleles on this region with Mol7 alleles would cause an increase of 0.8 cm in ear length.

## Ear Diameter

Chromosome 3 was identified containing QTL for EW in two environments (Table 9 and Figure 6). Estimates of additive effect were -0.1 in two environments. Estimates of dominance effects were 0.12 and 0.3 in Environments 1 and 2, respectively. Single marker analysis indicated dominance was the main source of variation. $B 52$ contributed the genes for smaller ear diameter. QTL were identified on chromosomes 1 in Environment 2 with large effect, enplaning $30.4 \%$ of the total phenotypic variation. Single marker analysis showed additive effects were the major source of variation for the region. Mol7 contributed genes for smaller ear diameter. Substitution of B 52 alleles on chromosome 3 with Mo17 alleles would cause an increase of 0.1 cm in EW . On chromosome 1 , additive and dominance effects were estimated as 0.1 and 0.4 in Environment 2. Cob Width

QTL for CW were detected on chromosomes 1, 3, 5 and 10 in both environments (Table 10 and Figure 7). QTL on chromosome 5 had the most
important effects on the inheritance of CW. Additive and dominance effects were estimated as 0.07 and -0.02 in Environment 1 and 0.05 and 0.05 in Environment 2. The region was identified by the same marker loci which cover a genome region of 16 cm in two environments and explained more than 16\% of the total phenotypic variation. Single marker analysis detected more dominance variation. Alleles for narrower cob were derived from Mol7. Two intervals were identified on chromosome 1 in Environment 2 and on chromosome 3 in Environment 1. The identification of two intervals was very possibly caused by the linkage between two intervals and a long genetic distance of one of the two intervals. The region on chromosome 1 derived alleles for narrow cob from Mol7 and the region on chromosome 3 from B52. The source for genetic variation varied dramatically. Significant dominance variation was detected for the region on chromosome 1 in Environment 1, but additive variation in Environment 2. Additive variation was the main source for CW for chromosome 3 in Environment 1 and dominance variation for Environment 2. QTL on chromosome 10 had consistent results in two environments. Estimates of additive and dominance effects were -0.06 and -0.04 in Enviromment 1, and -0.04 and -0.08 in Environment 2. Significant additive variation was detected in single marker analysis. Alleles for narrower cob were derived from B52. One region was identified on chromosome 9 in Environment 1 with significant dominance variation. Mol7 was the source for allele of narrower cob.

## Kernel Depth

The procedures for mapping failed to detect any QTL for KD in Environment 1. Three regions were identified on chromosomes 1, 3 and 9 in Environment 2 by interval mapping (Table 11 and Figure 8). The QTL with the largest effect was located to chromosome 1. Estimates of additive and dominance effects were 0.1 and 0.2 , respectively. Single marker analysis indicated the major region on chromosome 1 exhibited additive variation.

The other two regions had more dominant variation. Mol7 contributed genes for smaller KD in the major region on chromosome 1 , B52 to the other two minor regions.

## Single Marker Analysis vs. Interval mapping

Single marker analysis tended to identified the same regions as interval mapping (Appendix 10). Marker loci flanking regions with large LOD scores generally had lower probability values from single marker analysis. Examples included UMC67-UMC157 for GY and EN, UMC128-BNL15. 18 for KW, UMC51-UMC68 for KR, and NPI303U-PIO10.0033 for CW in Environment 1; BNL8.29-BNL15.18 for GY and EN, UMC128-UMC23 for KR in Environment 2. The above examples represented the situation that QTL were identified near the middle of the interval. Both markers had significant probability values. When QTL were identified near one of the two flanking markers, especially when the intervals were relatively large ( $>30 \mathrm{cM}$ ), the marker(s) closer to the QTL tended to be detected by single marker analysis only. For examples, UMC67 and BNL5.71 for GY and EN, UMC175 for EL in Environment 1, and UMC51 for GY and EN, ISU5 for EL, BNL8. 29 for KD in Environment 2. Marker $x$ Environment Interaction

Marker $x$ Environment interaction was tested for all regions containing putative QTL (Table 12). Significant marker by environment interaction was detected for the region detected on chromosome 1 and the region on chromosome 6 detected in Environment 2 for GY, on chromosome 1 for EN, on chromosome 1 for KW and EW. All regions identified for KD in Environment 2 exhibited significant Marker x Environment interaction. No interaction was detected for $K R, E L$ and $C W$.

The interval of UMC67-UMC157 was identified for EN in Environment 1 with LOD=5.2, but did not reach the threshold in Environment 2. Significant marker $x$ environment interaction was detected for the region. The similar observations could be obtained for other intervals such as

BNL8.29-BNL15.18 for EN and GY, and BNL15.18-UMC128 for KW. These intervals had one common feature: the intervals detected in one environment had relatively large LOD scores and led to relatively large discrepancies between the LODs in two environments. On the other hand, intervals exceeding the threshold in both environments had less interaction, such as the intervals of BNL7.71-BNL5.71 on chromosome 5 for CW, and BNL5.71-UMC51UMC68 on chromosome 5 for KR. Some intervals were identified with LOD slightly larger than the threshold (2.5) in one environment. There was not a large difference between the LOD scores in two environments. In this case, no significant interaction was detected, such as intervals of pllUMC85 on chromosome 6 for KW, and UMC114-BNL8. 17 for KR.

Examining the most likely position of QTL in the intervals, the interaction was more frequently detected by the marker closely linked to the QTL. QTL for EW in Environment 1 were determined to be closer to BNL15.18 than to BNL8. 29 and the interaction was more significant for BNL15.18. The same situation was found for the $K D$ in this interval. The interaction did not reach the significance level for several intervals, but the same trend was observed, such as UMC16-UMC175 for EN and UMC60-UMC165 for EL. The probability for marker by environment interaction was smaller for the locus closer to the QTL than for that of the probe further away from the QTL. This indicated the most significant difference detected by this test was directing to the most possible location of QTL. Therefore, the test might reflect the genetic basis of the variation.

Although it was not a general case for all the intervals identified, several intervals showed very different LOD scores in two environments, but no significant marker $x$ environment interaction was detected. Examples included UMC175-UMC5O for EL, Pl1-UMC85 and NPI268-UMC89 for EN, UMC67UMC157 for GY, and UMC128-UMC23 and BNL12.06-BNL5.62 for CW.

## Analysis of Marker x Environment Interaction

## Comparison of Results in Two Environments

The contribution of $G E$ to quantitative traits made it important to decide the testing environments for evaluation of GY. Rosielle and Hamblin (1981) reported results of evaluation in stress and normal condition(s) and concluded that the optimum approach would be evaluate material in the environments to which breeders aimed for production. Results from this study might provide some positive evidence for the suggestion by Rosielle and Hamblin (1981) at a new level. Different genetic factors were functioning, so different environments should be used to detect the effects of the factors, which might be reflected by different chromosomal regions.

Paterson et al. (1991) identified 29 QTL for fruit traits in tomato across three environments. Only four of the 29 QTL were identified in three environments, 11 were in two environments and 14 were identified in one environment. Stuber and Sisco, 1991 reported QTL for yield components across environment in several populations. The results indicated QTL with larger effects tended to be detected in all environments. The same kind results were reported by stuber et al. (1992). In the current study, different regions for yield and yield component traits were detected across environments. The differences can be distinguished as the following two cases. In the first case, QTL with major effects and relatively large LOD values were detected in both environments. Chromosomes with minor effects were detected in one of the two environments. For example, chromosome 1 and 5 for $E N$ and $G Y$, and chromosome 5 for $K R$ and $C W$ were detected in both environments with high LOD scores; chromosome 6 for $G Y$ was detected in Environment 2 with a LOD of 2.6. And there were some cases in which the region exceeded LOD score threshold in one environment, but just missed
exceeding the threshold in the other. In the second case, chromosome regions with high LOD scores were detected in one environment, not in the other. For example, the region on chromosome 1 for $K W$ had the highest LOD (8.8) in Environment 1; however, no QTL were detected on chromosome 1 in Environment 2. It could not be determined if the contrasting environments stimulated the expression of different QTL or some unremovable error caused the discrepancy, i.e. the source of the discrepancy was not clear.

At least two explanations were possible for each case. For case 1 , QTL of major effects functioned in all environments, but QTL with minor effects would only function in certain environments. All QTL worked like components of the phenotype of the trait. The major components behave like a constant and the minor components served as modifiers for changing environments. In the second explanation for case 1 , the same set of QTL functioned the same way genetically. The difference in detection was due to the relatively large environmental effects which reduced the sensitivity of the test in certain environment(s). In the first explanation for case 2, QTL with major effects functioned in different environments. There must be environmental factor(s) to direct the expression of QTL in certain environments. The second explanation for case 2, a large amount environment or/and genotype $x$ environment variation might mask the effect of some $Q T L$ in certain environment. In order this to happen, the environmental effects had to be very large. Case 1 is the situation breeders would have more use of the information.

## Analysis of Marker $x$ Environment Interaction

Analyzing environmental and environment $x$ genotype interaction effects has been an important subject of quantitative genetics and plant breeding. Partition of these components has had tremendous impact on breeding strategies (Comstock, 1963). Evaluation across environments is essential because repeatable ranking of genotypes is required. Hallauer et al.
(1988) summarized the definitions of stability and methods for GxE analyses. Selection of evaluation environments becomes important when breeders start evaluation programs (Lambert, 1984). One proposed method was to evaluate in environments typical of the target population of environments. The rationale under this method is that the genotypes should be evaluated in the environments to which they would often face the limiting factors. The other view is to evaluate genotypes in the optimum condition in order the genotypes to reach the highest potential. Rosielle and Hamblin (1981) conducted a theoretical study of grain yield in normal and stress conditions. The results supported that genotypes should be evaluated in the targeting environments. Johnson and Geadelmann (1989) conducted recurrent selection in a maize population in normal and moisture stress conditions. The results indicated grain yield gave superior results under irrigated conditions. Selection under irrigation was as effective as selection under dryland condition for increasing yield in moisture stress condition. The optimum allocation of test sites depends on the range of environments breeders are targeting. Several suggestions of selecting test environments were summarized in Hallauer et al. (1988).

The two growing seasons in which this study was conducted provided the opportunity of analyzing two contrasting environments, normal and water stress condition(s). The attempt to partition the marker $x$ environment component resulted in some potentially useful information (Table 12). The method was able to indicate the obvious difference between two environments for QTL detection. Overall, the number of significant interaction was not very high, which was the situation desired by breeders.

The reliability of the test for interaction between marker and environment, firstly, depends on a reliable linkage map (correct for the population involved in the study), especially when linked markers are involved. Secondly, accuracy of QTL location has direct consequence on
the test. If the QTL were not located correctly, the LOD scores and significant tests would not reflect the true genetic behavior of the chromosome(s) and the test based on a wrong assumption did not provide any correct information. This emphasizes the importance of accurate measurement in data collection and more information on experimental design and data manipulation.

## Experimental Design and QTL Location

Reliable location of QTL depended on accurate evaluation of quantitative traits. The sensitivity of distinguishing lines directly determined the ability to detect QTL. KD showed very low heritability (Table 2), mainly caused by the extremely low heritability in Env. 1 ( $h^{2}=6.3 \%$ ). The possible reasons for the low estimate was explained in the variation analysis: 1) one of the component traits for this trait (EW) had low estimate of heritability (43.4); 2) The unit of measurement did not provide sufficient accuracy for KD. The lack of differentiation among lines because of relatively high environmental variation in this population in Env. 1 was probably one of the main reasons for the failure to detect QTL. If an accurate measurement was not be able to been obtained, reliable location of gTL would not be reached.

The following points should be considered for accurate QTL location: 1) Experimental design should be considered carefully according to the materials involved; 2) Accurate measurements should be used to obtain the best possible data recorded; 3) Proper analysis procedures to maximize the usage of the information obtained from molecular techniques.

Correlation among Yield Components and QIL Location of Highly Correlated Traits

Highly correlated traits were reported to be mapped on the same chromosomes (Jarboe, 1993; Paterson et al., 1991). Traits considered in this study were grain yield and components of grain yield. Several high correlations were found. GY and EN were highly correlated ( $r=0.85$ ) in Environment 1. EN was one of the important contributors to GY. QTL for the two traits were identified on the same chromosomes by the same marker loci (Table 4 and 5). GY was highly correlated with two traits, EW ( $\mathrm{r}=0.72$ ) and $K \mathrm{CD}(\mathrm{r}=0.75)$ in Environment 2. The same chromosomes (1, 3, 5 and 6) were detected for GY and EW, and chromosome 1 and 3 were identified for KD; however, different marker loci represented the regions for these correlated traits. EW was highly correlated with KD Environment 1 ( $\mathrm{r}=0.76$ ) and Environment 2 ( $r=0.87$ ); however, QTL were not identified for $K D$ in Environment 1 so a comparison could not be made. In Environment 2 , chromosome 1 was detected for both traits by the same marker loci, and chromosome 3 was detected for the trait by different loci. The results suggested genetic factors contributed to the correlation among these traits. The regions identified by different marker loci on the same chromosomes for correlated traits suggested that correlation was attributable to linkage between loci for correlated traits. For the regions identified by the same marker loci for correlated traits, no conclusion could be made about the mechanism of correlation.

## Single Marker Analysis and Interval Mapping

Jarboe (1993) illustrated the relationship for single marker analysis and interval mapping. Stuber and sisco (1991) elucidated the usage of single marker analysis in the programg of marker-facilitated introgression for QTL in plant breeding programs. In this study, single marker analysis provided the opportunity to partition marker by environment interaction.

As more information available from basic studies describing the approximate position of $Q T L$, single marker analysis might be very suitable for the study of specific regions, environmental effects and the partitioning of different variation sources.

Table 1. Summary Statistics and Normality Tests of Grain Yield and Component Traits

| Trait | Env. | Mean | Range |  | c.v.A | Prob< $W^{\text {B }}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| ear |  |  |  |  |  |  |
|  | 1 | 18.4 | 3.0 - | 28.0 | 22.2 | 0.0001 |
| EN | 2 | 16.4 | 2.5 - | 25.0 | 30.0 | 0.0010 |
| Combined |  | 17.4 | 3.8 - | 23.5 | 23.2 | 0.0001 |
| g |  |  |  |  |  |  |
|  | 1 | 1338.8 | 200.0 - | 2563.0 | 30.3 | 0.7171 |
| GY | 2 | 1181.2 | 75.0 - | 2370.0 | 43.9 | 0.0121 |
| Combined |  | 1260.0 | 215.0 - | 2377.5 | 33.0 | 0.7237 |
| 9 |  |  |  |  |  |  |
|  | 1 | 62.2 | 40.9 - | 86.0 | 13.2 | 0.5697 |
| KW | 2 | 78.0 | 64.3 - | 94.9 | 8.1 | 0.0487 |
| Combined |  | 70.1 | 52.9 - | 87.0 | 9.1 | 0.3962 |
| rows |  |  |  |  |  |  |
| KR | 1 | 14.0 | 10.7 - | 17.6 | 7.9 | 0.3425 |
|  | 2 | 14.0 | 11.4 - | 17.2 | 7.4 | 0.4650 |
|  | Combined | 14.0 | 11.3 - | 17.3 | 7.2 | 0.3009 |
| 15 cm |  |  |  |  |  |  |
|  | 1 | 15.4 | 8.9 - | 19.4 | 9.9 | 0.5804 |
| EL | 2 | 15.5 | 11.3 - | 20.2 | 10.0 | 0.7789 |
|  | Combined | 15.4 | 11.7 - | 19.8 | 8.8 | 0.9532 |
| cm |  |  |  |  |  |  |
| EW | 1 | 3.8 | 3.3 - | 5.9 | 6.1 | 0.0 |
|  | 2 | 3.9 | 3.0 - | 4.3 | 6.2 | 0.0001 |
|  | Combined | 3.8 | 3.2 - | 4.8 | 4.8 | 0.0021 |
| cm |  |  |  |  |  |  |
| CW | 1 | 2.6 | 2.3 - | 3.5 | 5.3 | 0.0001 |
|  | 2 | 2.4 | 2.2 - | 2.7 | 4.9 | 0.0038 |
|  | Combined | 2.5 | 2.2 - | 2.9 | 4.5 | 0.2778 |
| cm |  |  |  |  |  |  |
| KD | 1 | 1.2 | 0.9 - | 1.9 | 14.6 | 0.0 |
|  | 2 | 1.4 | 0.8 - | 1.8 | 13.9 | 0.0003 |
|  | Combined | 1.3 | 1.0 - | 2.3 | 10.3 | 0 |

[^3]Table 2. Variation Analysis and Heritability Estimates for Grain Yield and Component Traits on $\mathrm{F}_{2: 3}$ Mean Basis

| Trait Env. |  | $h^{2}$ | $\sigma_{8}^{2}$ | $\sigma_{80}{ }^{2}$ | $\sigma_{0}{ }^{2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | 80.2 | (72.8-85.5) | 13.3** |  | 6.6 |
| EN 2 | 83.3 | (77.1-87.8) | 20.0*********) |  | 8.0 |
| Combined | 75.4 | (66.3-82.1) | 12.3** | $291.7^{* *}$ | 689.3 |
| 1 | 80.9 | (73.7-86.0) | 132804.1** |  | 642871.2 |
| GY 2 | 79.8 | (72.2-85.2) | 214395.7********* |  | 108825.9 |
| Combined | 75.0 | (65.7-81.8) | 129898.0** | $44302.5^{* *}$ | 84647.4 |
| 1 | 79.9 | (72.4-85.3) | $54.2{ }^{* *}$ |  | 27.3 |
| KW 2 | 78.1 | (69.9-84.0) | 31.1*** |  | 17.5 |
| Combined | 66.8 | (54.5-75.8) | 27.0** | 16.5** | 20.7 |
| 1 | 88.8 | (84.7-91.9) | 1.1******** |  | 0.3 |
| KR 2 | 75.6 | (66.5-82.2) | 0.8** |  | 0.5 |
| Combined | 84.8 | (79.1-88.9) | $0.9{ }^{\circ}$ | $0.1{ }^{* *}$ | 0.4 |
| 1 | 73.5 | (63.6-80.7) | 1.7*** |  | 1.2 |
| Combined | 76.7 | (68.0-83.0) | $1.8{ }^{* *}$ |  | 1.1 |
|  | 73.5 | (63.6-80.7) | $1.4 *$ | $0.4 *$ | 1.2 |
| 1 | 43.4 | (22.4-58.7) | 0.02*** |  | 0.06 |
| EW 2 | 71.7 | (61.2-79.7) | $0.04{ }^{\circ}$ |  | 0.03 |
| Combined | 35.0 | (10.8-62.6) | $0.01 * *$ | $0.02 * *$ | 0.05 |
| 1 | 55.6 | (39.2-67.6) | $0.01{ }^{\circ}$ |  | 0.02 |
| CW 2 | 67.2 | (55.0-79.1) | 0.01*** |  | 0.01 |
| Combined | 68.8 | (57.2-77.3) | $0.09 * *$ | 0.07 | 0.01 |
| 1 | 5.3 | ( 0 -23.2) | 0.001 |  | 0.07 |
| KD 2 | 67.2 | (55.0-76.1) | 0.01 |  | 0.01 |
| Combined | 7.9 | $(0-32.8)$ | $0.001 *$ | $0.01{ }^{*}$ | 0.05 |

[^4]Table 3. Phenotypic Correlation Coefficients among Yield Component Traits on the Basis of $F_{3}$ Line Means ${ }^{\wedge}$

|  | GY | KW | KR | EL | EW | CW | KD |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Env. 1 |  |  |  |  |  |  |
| EN | $\begin{gathered} 0.85 \\ (0.0001) \end{gathered}$ | $\begin{gathered} -0.40 \\ (0.0001) \end{gathered}$ | $\begin{gathered} -0.14 \\ (0.0927) \end{gathered}$ | $\begin{gathered} 0.40 \\ (0.0001) \end{gathered}$ | $\begin{gathered} -0.26 \\ (0.0010) \end{gathered}$ | $\begin{gathered} -0.33 \\ (0.0001) \end{gathered}$ | $\begin{gathered} -0.11 \\ (0.1534) \end{gathered}$ |
| GY |  | $\begin{aligned} & -0.20 \\ & (0.0103) \end{aligned}$ | $\begin{gathered} -0.07 \\ (0.4192) \end{gathered}$ | $\begin{gathered} 0.58 \\ (0.0001) \end{gathered}$ | $\begin{gathered} -0.01 \\ (0.9027) \end{gathered}$ | $\begin{gathered} -0.16 \\ (0.0434) \end{gathered}$ | $\begin{gathered} 0.04 \\ (0.6566) \end{gathered}$ |
| KW |  |  | $\begin{aligned} & -0.33 \\ & (0.0001) \end{aligned}$ | $\begin{gathered} -0.12 \\ (0.1249) \end{gathered}$ | $\begin{gathered} 0.20 \\ (0.0130) \end{gathered}$ | $\begin{gathered} 0.16 \\ (0.0480) \end{gathered}$ | $\begin{gathered} 0.17 \\ (0.0354) \end{gathered}$ |
| KR |  |  |  | $\begin{gathered} -0.07 \\ (0.3930) \end{gathered}$ | $\begin{gathered} 0.36 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.33 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.19 \\ (0.0197) \end{gathered}$ |
| EL |  |  |  |  | $\begin{gathered} 0.20 \\ (0.0125) \end{gathered}$ | $\begin{gathered} 0.08 \\ (0.2994) \end{gathered}$ | $\begin{gathered} 0.09 \\ (0.2582) \end{gathered}$ |
| EW |  |  |  |  |  | $\begin{gathered} 0.56 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.76 \\ (0.0001) \end{gathered}$ |
| CW |  |  |  |  |  |  | -0.05 |
| EN | $\begin{gathered} 0.89 \\ (0.0001) \end{gathered}$ | $\begin{gathered} -0.09 \\ (0.2600) \end{gathered}$ | $\begin{gathered} 0.20 \\ (0.0115) \end{gathered}$ | $\begin{gathered} \frac{\text { Env. } 2}{0.42} \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.61 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.11 \\ (0.1687) \end{gathered}$ | $\begin{gathered} 0.68 \\ (0.0001) \end{gathered}$ |
| GY |  | $\begin{gathered} -0.02 \\ (0.8062) \end{gathered}$ | $\begin{gathered} 0.23 \\ (0.0035) \end{gathered}$ | $\begin{gathered} 0.57 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.72 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.21 \\ (0.0082) \end{gathered}$ | $\begin{gathered} 0.75 \\ (0.0001) \end{gathered}$ |
| KW |  |  | $\begin{aligned} & -0.38 \\ & (0.0001) \end{aligned}$ | $\begin{gathered} 0.20 \\ (0.0134) \end{gathered}$ | $\begin{gathered} 0.13 \\ (0.1034) \end{gathered}$ | $\begin{gathered} 0.15 \\ (0.0631) \end{gathered}$ | $\begin{gathered} 0.07 \\ (0.3719) \end{gathered}$ |
| KR |  |  |  | $\begin{gathered} 0.17 \\ (0.0392) \end{gathered}$ | $\begin{gathered} 0.46 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.36 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.38 \\ (0.0001) \end{gathered}$ |
| EL |  |  |  |  | $\begin{gathered} 0.58 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.44 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.46 \\ (0.0001) \end{gathered}$ |
| EW |  |  |  |  |  | $\begin{gathered} 0.55 \\ (0.0001) \end{gathered}$ | $\begin{gathered} 0.87 \\ (0.0001) \end{gathered}$ |
| CW |  |  |  |  |  |  | $\begin{gathered} -0.13 \\ (0.5532) \end{gathered}$ |

[^5]Table 4. Regions for Grain Yield /plot Identified by Interval Mapping

| Chr. | Interval | Summary Scan |  | Multiple Loci Analysis |  |  |  | GA ${ }^{\text {A }}$ | LP ${ }^{\text {B }}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | peakLOD | $\mathrm{R}^{2}$ (\%) | Length Position ${ }^{\text {c }}$ Gene Effects ${ }^{\text {F }}$ |  |  |  |  |  |
|  |  |  |  | (cM) | (cM) | Add. | $\mathrm{g}^{\text {Dom. }}$ |  |  |
| Env. 1 |  |  |  |  |  |  |  |  |  |
| 1 | UMC67-UMC157 | 4.1 | 17.1 | 35.7 | 16.2 | - 194.6 | 123.4 | D | B52 |
| 3 | UMC16-ISU1 | 2.6 | 7.6 | 1.9 | 0.3 | - 18.2 | 348.6 | A | B52 |
| 5 | BNL5.71-UMC51 | 4.1 | 19.8 | 28.8 | 10.5 | - 147.0 | 419.6 | D | B52 |
|  | Sum | 10.8 |  | $\mathrm{R}^{2}=32.8 \%^{\text {d }}$ |  | LOD $=9.2{ }^{\text {E }}$ |  |  |  |
| Env. 2 |  |  |  |  |  |  |  |  |  |
| 1 | BNL8.29-BNLI5.18 | 4.2 | 17.7 | 29.9 | 8.0 | 280.0 | 473.8 | A | Mo17 |
| 3 | UMC175-UMC50 | 2.7 | 8.3 | 6.5 | 0 | -177.4 | -37.6 | D | B52 |
| 5 | UMC51-UMC68 | 4.6 | 17.5 | 25.2 | 7.6 | -292.2 | 403.0 | D | B52 |
| 6 | Pl1-BNL16.06 | 2.6 | 7.8 | 26.7 | 25.9 | 180.5 | 153.4 | D | Mol7 |
| Sum |  | 14.1 |  | $\mathrm{R}^{2}=48.8{ }^{\text {d }}$ |  | LOD=15.7 ${ }^{\text {E }}$ |  |  |  |

A Results from significance test by single marker analysis, at the significance level of $\alpha=0.001$, $A$ indicated significant additive variation, $D$ indicated significant dominant variation, $A / D$ indicated significant additive and dominant variation while additive variation had lower probability than dominance variation, D/A indicated significant additive and dominant variation while dominance variation had lower probability than dominance variation, and - indicated no significant additive and dominance variation was detected.
${ }^{B}$ LP indicated the parent contributing alleles conferring lower value for the trait
c Position of QTL relative to the loci defining the interval, denoted by the distance from the loci on the left in the interval
D Determination factor from multiple loci analysis.
E LOD score from multiple loci analysis.
F Estimates of gene effects from multiple loci analysis. Add. and Dom. indicated additive and dominance effects, respectively.

Table 5. Regions for Ear Number/plot Identified by Interval Mapping


A Results from significance test by single marker analysis, at the significance level of $\alpha=0.001$, $A$
indicated significant additive variation, $D$ indicated significant dominant variation, A/D indicated significant additive and dominant variation while additive variation had lower probability than dominance variation, $D / A$ indicated significant additive and dominant variation while dominance variation had lower probability than dominance variation, and - indicated no significant additive and dominance variation was detected.
B LP indicated the parent contributing alleles conferring lower value for the trait
c Position of QTL relative to the loci defining the interval, denoted by the distance from the loci on the left in the interval
D Determination factor from multiple loci analysis.
E LOD score from multiple loci analysis.
F Estimates of gene effects from multiple loci analysis. Add. and Dom. indicated additive and dominance effects, respectively.

| Chr. | Interval | Summary Scan |  | Multiple Loci Analysis |  |  |  | GA ${ }^{\text {A }}$ | LP ${ }^{\text {B }}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | peak <br> LOD | $\mathrm{R}^{2}$ (\%) | Length Position ${ }^{\text {c }}$ Gene Effectg ${ }^{\text {F }}$ |  |  |  |  |  |
|  |  |  |  | (cM) | (CM) | Add. | Dom. |  |  |
| Env. 1 |  |  |  |  |  |  |  |  |  |
| 1 | BNL15.18-UMC128 | 8.8 | 31.3 | 25.4 | 14.6 | - 7.2 | 0.14 | D/A | B52 |
| 2 | UMC61-UMC34 | 2.9 | 8.8 | 10.8 | 0.2 | 3.1 | - 5.0 | D | Mol7 |
| 6 | NPI280-ISU5 | 2.6 | 7.7 | 19.0 | 0.1 | 3.1 | - 4.2 | D | Mo17 |
|  | Sum | 14.3 |  | $\mathrm{R}^{2}=48.3 \%^{\text {D }}$ |  | LOD=1 | . $24{ }^{\text {E }}$ |  |  |
| Env. 2 |  |  |  |  |  |  |  |  |  |
| 2 | UMC78-NPI287 | 2.7 | 9.3 | 15.1 | 10.0 | 2.9 | - 2.2 | D | Mol7 |
| 3 | UMC60-UMC165 | 3.4 | 9.9 | 10.3 | 4.2 | - 3.1 | 1.4 | D | B52 |
| 6 | P11-BNL16.06 | 2.5 | 7.5 | 26.7 | 0.2 | - 2.3 | 1.4 | D | B52 |
|  | Sum | 8.6 |  | $\mathrm{R}^{2}=27.48^{\text {D }}$ |  | LOD=9.1 ${ }^{\mathrm{E}}$ |  |  |  |

${ }^{A}$ Results from significance test by single marker analysis, at the significance level of $\alpha=0.001$, $A$ indicated significant additive variation, $D$ indicated significant dominant variation, $A / D$ indicated significant additive and dominant variation while additive variation had lower probability than dominance variation, $D / A$ indicated significant additive and dominant variation while dominance variation had lower probability than dominance variation, and - indicated no significant additive and dominance variation was detected.
${ }^{\text {B }}$ LP indicated the parent contributing alleles conferring lower value for the trait
c Position of QTL relative to the loci defining the interval, denoted by the distance from the loci on the left in the interval
D Determination factor from multiple loci analysis.
${ }^{E}$ LOD score from multiple loci analysis.
F Estimates of gene effects from multiple loci analysis. Add. and Dom. indicated additive and dominance effects, respectively.

Table 7. Regions for Kernel Rows/Ear Identified by Interval Mapping


A Results from significance test by single marker analysis, at the significance level of a=0.001, A indicated significant additive variation, $D$ indicated significant dominant variation, $A / D$ indicated significant additive and dominant variation while additive variation had lower probability than dominance variation, $D / A$ indicated significant additive and dominant variation while dominance variation had lower probability than dominance variation, and - indicated no significant additive and dominance variation was detected.
${ }^{\text {b }}$ LP indicated the parent contributing alleles conferring lower value for the trait
c Position of QTL relative to the loci defining the interval, denoted by the distance from the loci on the left in the interval
D Determination factor from multiple loci analysis.
${ }^{\text {E }}$ LOD score from multiple loci analysis.
F Estimates of gene effects from multiple loci analysis. Add. and Dom. indicated additive and dominance effects, respectively.

Table 8. Regions for Ear Length Identified by Interval Mapping

| Chr. | Interval | Summary Scan |  | Multiple Loci Analysis |  |  |  | GA ${ }^{\mathbf{A}}$ | $L \mathbf{P}^{\mathbf{B}}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | peak <br> LOD | $\mathrm{R}^{2}$ (\%) | Length (CM) | $\begin{aligned} & \text { Position } \\ & 1 \quad(\mathrm{~cm}) \end{aligned}$ | Gene Effects ${ }^{\text {F }}$ |  |  |  |
|  |  |  |  |  |  | $(\mathrm{cm})$ |  |  |  |
| Env. 1 |  |  |  |  |  |  |  |  |  |
| 3 | NPI457-UMC16 | 2.6 | 17.9 | 32.7 | 24.4 | -0.4 | 1.4 | - | B52 |
| 3 | UMC60-UMC165 | 3.6 | 10.4 | 10.3 | 0.2 | 0.5 | 1.4 | - | Mol7 |
| 3 | UMC175-UMC50 | 4.0 | 11.4 | $6.5$ | $0$ |  | 0.6 | D/A | B52 |
|  | Sum | 10.2 |  | $R 2=27$ | $0 \% \mathrm{D}$ | $L O D=8$ |  |  |  |
| Env. 2 |  |  |  |  |  |  |  |  |  |
| 1 | BNL8.29-BNL15.18 | 2.6 | $12.5$ | 29.9 | 10.1 | 0.4 | 1.6 | A | Mo17 |
| 3 | UMC26-UMC175 | 4.4 | 14.0 | 10.3 | 7.3 | $-0.8$ | 0.4 | D/A | B52 |
| 5 | BNL10.06-BNL7.71 | 3.1 | 9.5 | 3.4 | 1.7 | 0.6 | 0.8 | A/D | Mo17 |
| 6 | ISU5-P11 | 4.2 | 12.9 | 4.5 | 2.6 | -0.7 | 0.2 | D | B52 |
|  | Sum | 14.1 |  | $\mathrm{R} 2=43.7 \%^{+}$ |  | LOD=15.5 ${ }^{++}$ |  |  |  |

A Results from significance test by single marker analysis, at the significance level of a=0.001, A indicated significant additive variation, $D$ indicated significant dominant variation, $A / D$ indicated significant additive and dominant variation while additive variation had lower probability than dominance variation, $D / A$ indicated significant additive and dominant variation while dominance variation had lower probability than dominance variation, and - indicated no significant additive and dominance variation was detected.
B LP indicated the parent contributing alleles conferring lower value for the trait
c Position of qTL relative to the loci defining the interval, denoted by the distance from the loci on the left in the interval
D Determination factor from multiple loci analysis.
E LOD score from multiple loci analysis.
F Estimates of gene effects from multiple loci analysis. Add. and Dom. indicated additive and dominance effects, respectively.

Table 9. Regions for Ear Diameter Identified by Interval Mapping


A Results from significance test by single marker analysis, at the significance level of $\alpha=0.001$, $A$ indicated significant additive variation, $D$ indicated significant dominant variation, $A / D$ indicated significant additive and dominant variation while additive variation had lower probability than dominance variation, D/A indicated signiricant additive and dominant variation while dominance variation had lower probability than dominance variation, and - indicated no significant additive and dominance variation was detected.
${ }^{B}$ LP indicated the parent contributing alleles conferring lower value for the trait
c Position of QTL relative to the loci defining the interval, denoted by the distance from the loci on the left in the interval
D Determination factor from multiple loci analysis.
${ }^{E}$ LOD score from multiple loci analysis.
F Estimates of gene effects from multiple loci analysis. Add. and Dom. indicated additive and dominance effects, respectively.

Table 10. Regions for Cob Width Identified by Interval Mapping

| Chr. | Interval | Summary Scan |  |  | Multiple Loci Analysis |  |  | GA ${ }^{\text {A }}$ | LP* |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | peak | $\mathrm{R}^{2}$ (\%) | Length (cM) | Position ${ }^{\text {c }}$ | $\frac{\text { Gene Effects }}{\text { Add. }} \text { Dom. }$ |  |  |  |
|  |  | LOD |  |  | (cM) |  |  |  |  |
| Env. 1 |  |  |  |  |  |  |  |  |  |
| 1 | NPI1429-UMC67 | 2.9 | 8.5 | 9.6 | 0.2 | 0.04 | -0.002 | D | Mo17 |
| 3 | NPI250-NPI457 | 2.5 | 9.2 | 56.7 | 42.9 | 0.02 | 0.06 | A | Mo17 |
| 3 | UMC16-ISU1 | 3.1 | 9.2 | 1.9 | 0 | - 0.05 | -0.06 | A | B52 |
| 5 | BNL7.71-BNL5.71 | 4.9 | 16.4 | 16.4 | 7.0 | 0.07 | -0.02 | D | Mol7 |
| 9 | UMC153-UMC114 | 2.6 | 7.5 | 1.2 | 0 | 0.02 | 0.10 | D | Mol7 |
| 10 | NPI303U-PIO10.0033 | 3.1 | 9.0 | 3.0 | 0 | - 0.06 | -0.04 | A | B52 |
|  | Sum | 19.1 |  | R2 $=$ | $43.2 \%^{\text {D }}$ | LOD=16 |  |  |  |
| Env. 2 |  |  |  |  |  |  |  |  |  |
| 1 | UMC128-UMC23 | 4.1 | 14.9 | 8.6 | 3.4 | 0.04 | 0.08 | A | Mol7 |
| 1 | BNL15.06-BNL5.62 | 4.8 | 67.9 | 47.3 | 22.8 | 0.01 | 0.2 | D | Mo17 |
| 3 | NPI457-NPI250 | 2.5 | 68.9 | 32.7 | 26.3 | - 0.03 | -0.02 | D | B52 |
| 5 | BNL7.71-BNL5.71 | 4.1 | 16.7 | 16.4 | 8.5 | 0.05 | 0.06 | D/A | Mol7 |
| 10 | NPI232-NPI287 | 2.6 | 9.2 | 17.5 | ${ }^{3.7}$ | -0.04 | ${ }^{-0.08}$ | A | B52 |
|  | Sum | 18.1 |  | $\mathrm{R} 2=$ | $55.68{ }^{\text {D }}$ | LOD=12 |  |  |  |

^ Results from significance test by single marker analysis, at the significance level of $\alpha=0.001$, $A$ indicated significant additive variation, $D$ indicated significant dominant variation, A/D indicated significant additive and dominant variation while additive variation had lower probability than dominance variation, $D / A$ indicated significant additive and dominant variation while dominance variation had lower probability than dominance variation, and - indicated no significant additive and dominance variation was detected.
${ }^{-}$LP indicated the parent contributing alleles conferring lower value for the trait
c Position of QTL relative to the loci defining the interval, denoted by the distance from the loci on the left in the interval
D Determination factor from multiple loci analysis.
E LOD score from multiple loci analysia.
F Estimates of gene effects from multiple loci analysis. Add. and Dom. indicated additive and dominance effects, respectively.

Table 11. Regions for Kernel Depth Identified by Interval Mapping

| chr. | Interval | Summary Scan |  | Multiple Loci Analysis |  |  |  | GAA | LP* |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | peak LOD | $\mathrm{R}^{2}$ (\%) | Length (CM) | Position (CM) | Gene Effects ${ }^{\text {F }}$ |  |  |  |
|  |  |  |  |  |  | (cm) |  |  |  |
| Env. 2 |  |  |  |  |  |  |  |  |  |
| 1 | BNL8.29-BNL15.18 | 5.8 | 32.5 | 29.9 | 16.0 | 0.1 | 0.2 | $D / A$ | Mo17 |
| 3 | UMC175-UMC50 | 2.5 | 8.2 | 6.5 | 3.4 | $-0.06$ | 0.08 | D | B52 |
| 9 | PIO10.5-CI | 3.2 | 11.3 | 11.8 | 10.0 | - 0.07 | 0.06 | D | B52 |
|  | Sum | 11.5 |  | R2 $=$ | .8\%D | LOD=11 |  |  |  |

A Results from significance test by single marker analysis, at the significance level of a=0.001, $A$ indicated significant additive variation, $D$ indicated significant dominant variation, $A / D$ indicated significant additive and dominant variation while additive variation had lower probability than dominance variation, $D / A$ indicated significant additive and dominant variation while dominance variation had lower probability than dominance variation, and - indicated no significant additive and dominance variation was detected.
${ }^{\text {s }}$ LP indicated the parent contributing alleles conferring lower value for the trait
c Position of QTL relative to the loci defining the interval, denoted by the distance from the loci on the left in the interval
D Determination factor from multiple loci analysis.
E LOD score from multiple loci analysis.
F Estimates of gene effects from multiple loci analysis. Add. and Dom. indicated additive and dominance effects, respectively.

Table 12. Analysis of Marker x Environment Interaction


Table 12 (continued).


Table 12 (continued).


Table 12 (continued).

${ }^{\wedge}$ LOD $D_{1}$ and $L O D_{2}$ represented LOD scores in Env. 1 and Env. 2; Pr. mxE>F indicated the probability of marker by environment interaction $>F_{a} ;$; connected the marker loci identifying the intervals.

* indicated RFLP marker closer to the QTL in the interval.

C1


C2


C3


C4


Figure 1. Plot of LOD Score for Grain Yield/plot Detected for Each Environment.
The X axis represents the chromosome with vertical bars indicating the distribution of RFLP loci. The $Y$ axis is the scale of LOD scores.

C5


C8


C7


C8

C9


C10


Env. 1 Env. 2

C1


C2


C3


C4


Figure 2. Plot of LOD Score for Ear Number/plot Detected for Each Environment.
The $X$ axis represents the chromosome with vertical bars indicating the distribution of RFLP loci. The $Y$ axis is the scale of LOD scores.


C6

$C 7$


C8


C9

C10


Env. 1
Env. 2

C1


C2


C3


C4


Figure 3. Plot of LOD Score for 300-kernel Weight Detected for Each Environment. The x axis represents the chromosome with vertical bars indicating the distribution of RFLP loci. The $Y$ axis is the scale of LOD scores.


C8


C7


C8


C9



Env. 1
_Env. 2

C1


C2


C3


C4


Figure 4. Plot of LOD Score for Kernel Rows/ear Detected for Each Environment.
The $X$ axis represents the chromosome with vertical bars indicating the distribution of RFLP loci. The $Y$ axis is the scale of LOD scores.




C3


C4


Figure 5. Plot of LOD Score for Ear Length Detected for Each Environment.
The $X$ axis represents the chromosome with vertical bars indicating the distribution of RFLP loci. The $Y$ axis is the scale of LOD scores.

C5


C8


C7


C8


C9


C10


Env. 1
-_-__Env. 2

C1


C2


C3


C4


Figure 6. Plot of LOD Score for Ear Diameter Detected for Each Environment.
The $X$ axis represents the chromosome with vertical bars indicating the distribution of RFLP loci. The $Y$ axis is the scale of LOD scores.

C5


C8


C7


C8


C9


C10


Env. 1
Env. 2

C1


C2


C3


C4


Figure 7. Plot of LOD Score for Cob Width Height Detected for Each Environment.
The $X$ axis represents the chromosome with vertical bars indicating the distribution of RFLP loci. The $Y$ axis is the scale of LOD scores.

## C5



C8

C7

C8


C9


C10


Env. 1
Env. 3

C1


C2


C3


C4


C5


Figure 8. Plot of LOD Score for Kernel Depth Detected for Each Environment.
The $X$ axis represents the chromosome with vertical bars indicating the distribution of RFLP loci. The $Y$ axis is the scale of LOD scores.

C8

C7


C8


C9



Env. 1
Env. 2

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## SUMMARY DISCUSSION


#### Abstract

Restriction fragment polymorphisms (RFLPs) have been shown potential in plant breeding programs. One aspect is the dissection of quantitative loci. The inheritance of quantitative traits is more complicated than that of the traits controlled by a single or a few genes. Traditional breeding has depended on the results from quantitative genetics studies which estimated the pooled effects of multiple loci. As most traits of economic importance are quantitative traits, dissection of individual loci is of interest to breeders.

In this study, one hundred and thirteen genomic and CDNA clones were included to construct a linkage map for maize genome. On the basis of the linkage map, $150 \mathrm{~F}_{2: 3}$ lines were evaluated for traits in various experiments over environments. The same $150 \mathrm{~F}_{2: 3}$ lines were analyzed in the laboratory for RFLP scores. The data were used to detect the putative QTL for the measured traits in the population by two approaches, interval mapping and single marker analysis in a linear model.

In general, regions with large effects tended to be detected in more than one environment, regions with small effects were detected in one environment only. There were exceptions; however, the general trend was obvious. This might reflect that the regions with major effects function for the trait expression in all environments, the regions with minor effects only function in certain environment(s). However, the results could not exclude the possibility of different detection levels of the analysis in different environments, as the environments were not identical and some environment(s) showed more environmental effects than others. This results suggested more studies should be done on sample size for research using molecular markers to locate QTL, to partition variation to interpret the effects of different components and the reduction of


experimental error.
Results from interval mapping and single marker analysis were similar to each other as expected because the two approaches reflected the same facts. As long as markers were evenly distributed in the linkage groups and proper threshold level (in interval mapping) and significance level (in single marker analysis) were selected, either method would be sufficient to detect QTL for traits of interest. However, each method did have some specific features. Interval mapping indicated the location of QTL by the interval and the relative position of the QTL to the markers was defined. Single marker analysis, on the other hand, can only indicate the linked marker(s), not the relative location of the QTL to the marker(s). Single marker analysis can provide a significance test for additive and dominant variation. It is important for breeders to realize that a saturated linkage map is one of the key issues for QTL mapping.

Dominance variation was detected in most cases. This might be contributed to the maximized linkage disequilibrium in $F_{2}$ population.

A partition of marker by environment interaction was conducted for the yield component traits. This partition was able to detect the variation and quantify the interaction. As this interaction was important for breeders, especially for traits like yield, partition of marker by environment interaction and finding a practical way to use the information in breeding programs.

QTL location required accuracy of measurements. Accurate mapping provided information for breeding programs such as marker assistant selection programs (Stuber and Sisco, 1991). On the other hand, a theoretical simulation had indicated that molecular marker information would significantly increase the efficiency of traditional selection program for the traits with relatively low heritability (narrow sense, the proportion of additive variation) (Lande and Thompson, 1990). This may be


#### Abstract

very important in animal breeding where in some circumstance, relative information was the only source of performance, construction of index with marker information became highly informative. In plant breeding, high heritability could be reached by family evaluation and selection. The efficiency of marker-assistant selection might not be as high as in animal breeding. Reduced cost of marker analysis and field evaluation became important. For plant breeder to be able to use molecular information, it is important to improve the laboratory process, especially reducing the cost; however, another very important aspect that can not be at neglected is the process of the information. More efficient design and analysis methods need to be developed by the joint effort of plant breeders, statisticians and molecular biologists, since molecular data have some special features different from data in a conventional breeding programs.


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## APPENDIX 1. ABBREVIATIONS

| Abbreviation | Term |
| :---: | :---: |
| RFLPs | Restriction Fragment Length Polymorphisms |
| ECB | European Corn Borer |
| 1ECB | First Generation European Corn Borer |
| 2ECB | Second Generation European Corn Borer |
| ANOVA | Analysis of Variance |
| CRBD | Complete Random Block Design |
| GDD | Growing Degree Day |
| TS | Tasseling Date (days after June 30) |
| ATS | Tasseling Date (GDD) |
| SL | Silking Date (days after June 30) |
| ASL | Silking Date (GDD) |
| PT | Plant Height |
| ET | Ear Height |
| EN | Number of Ears per Plot |
| GY | Grain Yield per Plot |
| KW | 300 Kernel Weight |
| KR | Number of Kernel Rows per Ear |
| EL | Ear Length |
| EW | Ear Diameter |
| CW | Cob Width |
| KD | Kernel Depth |

APPENDIX 2. ENZYME-CLONE COMBINATIONS USED IN RFLP ANALYSIS Egori

| UMC23 | NPI234 | BNL5.62 | UMC33 | UMC165 | UMC53 |
| :--- | :--- | :--- | :--- | :--- | :--- |
| AGP2 | UMC98 | ISU4 | BNL1.297 | NPI565 | UMC60 |
| BNL8.15 | UMC121 | UMC26 | BNL7.08 | UMC50 | BNL7.65 |
| UMC31 | NPI203 | P2mISU033 | UMC166 | BNL7.43 | BNL10.06 |
| BNL6.25 | BNL5.02 | UMC51 | UMC67 | BNL8.33 | PIO10.0016 |
| NPI280 | UMC62 | DEK326 | BNL13.24 | BNL16.06 | UMC116 |
| NPI268 | BNL9.44 | CI | UMC114 | UMC20 | UMC153 |
| UMC64 | BNL5.71 |  |  |  |  |

## HindIII

| BNL12.06 | UMC157 | BNL8.29 | NPI429 | UMC78 | UMC5 |
| :--- | :--- | :--- | :--- | :--- | :--- |
| UMC88 | UMC34 | UMC131 | BNL6.20 | BNL15.20 | UMC32 |
| UMC39 | ISU1 | UMC16 | BNL5.37 | BNL15.27 | UMC15 |
| UMC158 | PIO10.0025 | BNL5.46 | BNL15.07 | NPI292 | UMC90 |
| UMC104 | BNL7.71 | UMC68 | UMC70 | UMC21 | BNL3.03 |
| UMC85 | BNL15.40 | BNL8.37 | UMC80 | BNL8.39 | BNL15.21 |
| BNL9.08 | UMC103 | BNLB.26 | BNL14.2 | UMC81 | PIO10.0005 |
| PIO10.0033 | NPI303U | NPI232 | BNL10.17 | PII |  |

EcoRV

| BNL15.18 UMC128 <br> NPI220 | UMC58 UMC61 NPI287 | BNL14.07 | UMC89 |
| :--- | :--- | :--- | :--- | :--- | :--- |

APPENDIX 3. RELP SCORES FOR B52XMO17 $\mathrm{F}_{2: 3}$ LINES

Marker
RFLP Scores for Line 1 - 85

BNL12. 06
UMC157
UMC161
UMC2 3
NPI234
BNL5. 62
BNL15. 18
BNL8. 29
UMC128
UMC58C
UMC33
UMC164
NPI1429
UMC61
UMC78
NPI287(1)
NPI287(2)
UMC5
UMC53
UMC88
AGP2
UMC34
UMC98
UMC135
UMC131
BNL6. 20
BNL1. 297
NPI565
BNL15. 20
UMC60
BNL8. 15
UMC32
UMC39
UMC175
UMC121
UMC26
BNLT.O8(1)
BNL7.03(2)

ННВНННННАННВВВНАННВАНННАВАНВАААНААННАНВНВВВВАВААНННАВАНВАНННААВННВВНВВАННАААНАНВНАВ HBHAHBAHHABABBHHAHBHHBBABBHHHAHHAHBHBHHHHBBAHBAHAHHHHAAHAAHBAABAHBHHBHAHHAHAAAHABHBA НННАНАНННННННАННВВАНАНАННААВВНАНВНННВВННВНВВАНННВАВАААВВНННННААНННННВААННВААННАВННHH -НАНВНННВВНАВНАНВАВННАНН-НВННАНННННАВАНННННАНВВНВВНННВНАВВВНННВВННННННАНВАННВВНАВВНА АВНАНННАAHBHBBBHAABHHHBABHHBHAHHAHBHHHHHHB-HHBAAHHHHBAHHAAHBAA-HH-HH-H-HAAHAAA-HHHBA АНННВАНННННВВВНАНВАНАААНВАВВАНННННННННВАВВНВАНННВНННННАВНВАННАНААНВНВВАНАННавННВАНАА ННВННАНВН-НННАННВААННА--ННННАНВНННН-НН-ННАНАНВНАНВВВНВННННВНВА-ВННННВВ-ННАНАНВАННВВА ННВАНАНННННННААННААННАННВНАНААВННАНАННААННННВНААННВННВННВНВНВНВВВВННВНВННАНАННННАННН ННАНВНННВВННВААНВННННА-АННВННАНАННН-НННННАНАНВВНВВНННВНАВВВ--- ВВНННННААНАНАВВНАВВНА ВНННВВННВАНАВВАВВНВННННННВВНААНННВН-ВАНННВНАНВННВВНВВВВНВНННАНВВНННННААВВАНННННАВВНН ВНАНВНННВВНАВНАНВНВННАННННВННАНННННАВАННВН-АНВВНВВННВВНАВ--НННВВННННННАНВАННВВНАВВНА АННАНННННННАНААААНАНННАННААНВНАНВНННВНННННВВАННАВНВАAABBHHBHAAHABBHABHAHBHAHHHAHHBBH ВННHHBABBAHAH-ABBHHHHHBHHBBHAAHHHHHABAHHHBHAABHHHBHHHHBHBHHBAHBHHHHHHAABBAHHAAHABBAH ННВАНВННН-АНАННВАНННВАВНВНАНАНАВННВ-НВВННА-НННННННАНн-НННВВАННАННАНААН-ВВВНННААНННВА
 ННВАНВНННННАННВА--Н--ВНВНАНАААВННВ-НВВННАННАНННННАННАНННВВАННАННАНАНН-ВВВНННААНННВА НВННАВННН-АНВАВНН--H--ВНННВННННВНВН-ВНВАНАВАННАНННАНАНВНННННВАННВНННВВ-АНВНННННННАНН -ВНАНВАНВННВНАВАВННHBHBHBAHBAHHHHBBHHBHHHHHHAHAAHHHAHABHHBB-HH-HHABHHHBHBBHBAHHBHHBB НАААНННАНННВАНААННАНВ-ВННВАННАНВАНВВННВВВА-ВНВННВНАВННННВАННААНВНВНАВАВНННННВНВаНВВН ВВНАНВАВВННHHABABHHHBBBHBAHHAHHHHBBHHBHHHHHBAHAAHHHHBABHHBBHHH-HAABHBHBHBBHBAHBBHABH НВНАНВАНННАННАВАННННВНВНВАНВАВНННННННВННННННАННнНННАН-НННВВННААННАННННВНВВНВААнннНВ НВВАНВНННВАННАНВАНННВАВНВНАНАВАВННВННВВННАННННННННВАНННННВВАВНАННАНАННННВВНВНААНнНВН НВНАНВАНВННВНАВАВНННВНВНВННВААНННВВННВНHHH-HHH-AHHH-HABH-BBHHH-HHABHHHBHBBHB-HHBHHBB HBBHHBAHH-AHHABHAHHHBHBBBAHBABHHHHHHHBHHHHHHAHHHHHHAHAHHHBBHBAAHHAHHHHAHBBHBAAHHHHBH
 ВВНННВНВВННннАВАВНННВВННВНННАННННВВАВНННННАНАНААННННВАВНННВА---- НННАВНВНВВНВАНВВААВН ННААВННННННАВНННАНАНННАНВНАНВННАВНВНВАВННННВННААВННАНННАННВННАААНВНАННАВНННВНВННВНВН

 Н-НАНННННННАВААААВАНННАНННААВНААВНННВНННННВВАНААВНВАННВВННВННАНАВВНАНВАВВНАНННННВВВН ВНАНВВН-ННННННННННАНАВАВАВНННАНННА--ВНАН- - -HHAHBBHAAHHHHHHHHHAHABABABBHHAHHHAHHBHAHAABHHHBHHHAHHAABHHHHHH--HAHHBAHBHABHHAHB-H--
 ННААВННННННАВНННАНННННАНВНАНВНВАВНВНВАВННА-ВННААВННАН-НАННВННАВАНВНАНВАВНННВНВАНВНВН НННННАНААНННВНВНННАНАААННННННААВННВНВНАНААВННАННВННННАНВВННВНННАНАНАВННВНННАНННВАНАА ВННАНАННННААНАААННАНАНАННААВННАНВНННВНННННВВАННHBABAAHBBHHHHHA-HH-HHBAAHHBAHBHHBHBHH BAAHBHHHBBBABBABBABHHAAHHHBAHAHHBHHABAHHHH-AHBBHHBHBBBHHBBHHHHBBHAHHAHAHBAHHBBHABBAA АВВНННВВННАННАА--АНАВНАННАНАНАНВННАВННВВНА-НВНННВННАААВВА-ВВАНААВААВВНВААААААННВАНАН

UMC16C3
BNL5. 37
UMC50
BNL15. 27
BNL7. 65
UMC158
UMC31
UMC15
PIO10. 25
BNL5. 46
BNL15.07
NPI292
NPL203
UMC90
JC162
UMC166
UMC104
BNL7. 43
BNL10.06
BNL6. 25
BNL7. 71
BNL5. 02
UMC5 1
UMC67
UMC68
BNL8. 33
PIO10. 16
UMC70
NPL280
UMC21
BNL3. 03
UMC62
UMC85
BNL15. 40
DEK326
BNL13. 24
BNL14.07
BNL8. 37
BNL16.06
UMC110
UMC116
UMC80
BNL8. 39

АНААВННННННАВНННАНННННАНВНАНВННАВНВНВАВННАНВННААВННАНВНАННВННАВАНВНАНВаBHHHBHBAHBHBH ВННАНННННННАНАААННААА-АННААНВНАНВНННВННННН-ВАНННВНВААНВВНННННА-НВАВАВААННВАНННАННВНН ВННАНАНННННАВАННВНАНАНАННААВВНАНВНHHBBHHBHBBAHHHBAHAAHBBHBHHHHAHHHHHBAAHHBAAHHBHHHH HBHAHHHHHHBHBBHHBBHHHBHBBHBHHBHBABAHHHHBHAHBBAABBHAHBHHHHAHAHABABHBABHHBBHHHHAAABBAA НАВННННВНННHHBHHHHHHAHBHBBBHABHBBHHHAABHAHHHAHHBHHHHHHBBAHHHHHHHBBHHBHHHHHHBHAHHAHBA НАВННННВНННННВННННННАНВННВВНАВНВВНННААВНАН-НАННВННННННВВАННнННАНВВННВННННННВНанНАНВА НННААНННННВВВВНННВВННННВВНННННННННАННАВНААНВНААНВНАНАНВННННАААВВВНННВННВВННВНАААВНАА НАВННННВНННННВННННН-АНВННВВНАННВВНННААВНАНАНАННВННННнНВВАНнHH--Н-ВННВН-ННАНВВАННАНВН НАВННННВНННННВННННННАНВННВВНАННВННННААВНАНАНАННВННННнНВНАНннННАНВВННВННННАНВНАННАНВН НВНАННННННВНВВНННВВННHHBBHHHHHHBHHAHHAHHHAHBHAAHBHAHHHBAHHHAAABBBHBHBBHBBHHBHAHABHAA
 НВВНН-НВНННННВННННННАНВНВНВ-А-НВВНННААННАН-ВАННВННННННВВАНННННННВВНН- НННННН- - ААНА - -А
 ННВАВНАННННАНВАННАНННААНВВННАНАВАННВНВВННАННАНННВНВНННААННВНННВВНАНААННАНННННВНААНАН НННАННАННННАНВАНВАННВАННВВННАНАВАННННВВНННАНННАНВНННАНАААВННННВВНАНАННАААННАНННААААА НННАННАННННАНВАНВАННВАННВВННАНАВАН--НВВНННАНННАНВНННННААА-ННННВВНАНАННАААННАНННААААА ВАНВННАНАВВНВВНВВАНННАНВНАННВВННВНААНВАВАВНВВНВНВААНВНАНВНАН---НАНННВНВННННВАВНННННА ННААННАННННННВА--АНННАННВВННННАВАННННВВНННАНВНАНВНННАНАААВННННАННАААННАААННАНННААНАА ННААННАННННННВАНВАНННАННВВННННАВАННННВВНННАНВНАНВНННАНАААВННННАННАААННАААННАНННААНАА НАННННАННННАНВАННАННННАНВНННАНАВНННВНВВННАНВННВ-ВАВВННААННВННАВВАННННАНАННННВВААННВН ВНААННАННННННВАНВАНННАННВНННННАВАННННВВНННАНВНАНННННАНАААВННННАННАААННАААВНННННАННАА НННАННАННННАНВАНВАННВАННВВННАНАВАННННВВННН-НННАНВНННАНАААНННННВВНАНАННАААННАННННАААА ВАААНВНННВНННВННВАНННАННВННАННАВАНАННВННАННННННАННАННАВАННАНННВННААНННННВВНННННВНННА НННАНВАВНАВАВВАВВНВНННВННВВНААННАННАВНННАВНААВННВВАВВНВНВАНВАНВННННННААВВАННААНВАНАН HAAAHBAHABBHBBHHHAHHBAAHBAHHHBHBHHAHHBHHAHHHBBBHHHAHBHHAHHAHHHBHAAHHHAAAHBHBABHHHBHA НАНННВНАНННАННАННВННННААВНАНАННВННВВНВВННАВВННВНВАНВННННВНВННАВНАННННАНАНННнHHHHHHHH АНННАВННВННННАВВННВНАНВНННН-НВНВНВ-АВНВААА -НННННННАНА - ВВНННННННН- НВВН-АНВНАВНВННННН НАВВНВНВВАННННВНННАНАВННННАВННННВНННВНВНАВВАВВВНННААНВНАВННВНН--НАНАННАВНННАНАННВАНА

 ВНАНВНВВВНННВННННВННННННВАН-НННАННННАВННВН-ABAHBHHHHBBHBBAHBAA-BHHHBHB-BHAHAHHB--HAA
 ННАНВНВВНВННнНнВННННННВНВННННАВАВННВАВННВН-АНАНВАНВВВВНВННВНАННВНнНВННАВНАААННВАНнНВ ВНВВВВНННННННННАНВАНННННВНВВНАННННННВАНННННВВАВАВННВНнННАВНВННВННАВНННВВНААВВННННАВА ННАВНВНВННАНАНННННВННВННННВННААННВВНВАНННН-АНВВНННВНАВННАННВВННННАННННННННАНВНАВВННН HHABHHHBHAHHHHHAHHHHHHHHBHBHHAHHHBBHBAHHHHHBHHBHHHBHABHAHBHBHHBHHAHHHBBHHHABBHABBAHA ННАВНВНВННАНАНнНнHBAHBHHHHBHHBBHHBB-ВАНHHHHAHBBHHHBHHBHHABHBBHHHHAHHHBHHHBAHBHABBBHH ННАВНВНВННАНАННАННВННВННННВННААННВВНВАНННННННВВНННВНАВНННВНВВН-ННАНННВНННВАНВНАВВВНН ННВАНВНВАНАНАВННВНВАНВННННВННААНННННВНАА-Н-ААВВНННННННВНАННАВ--АНВАВНВ-АНВННВНННВВАН ННАВНННВНННННННАННАНННННВНВННАНННВВНВАНННННВННВНННВННВНАНВНВННВННАНННВВНННАВВННВВАНА ВНВВНВННАННННННАННАНННННВНВННАННННВНВАНННHHВНАВАНННВНВНААННВННВННАВННВВВННАВВННВВАВА НАНННВНВННАНАВННВНВННВННННВННААНННВНВНАААН- ААВВАННВНННВНАВАНВН-АННАННВ ННАВНВНВННАНАНННАНВАНВННННВННВВННВВНВННААН-ААВВАННВНАВННАВННВННАНАНННВНННВАНВНАВВВНН

BNL15.21
BNL9. 08
NPI268
UMC89
NPI220
BNL9. 44
UMC103
BNL8. 26
BNL14.28
cIC9s
UMC114
UMC20
PIO10. 5
UMC81
UMC153
PIO10.33
UMC64
NPI303U
NPI232
BNL10. 17
BNL5. 71
BNL3. 06
NPI560
PURPLE
UMC11
NPI457
NPI250
PHI10. 17
PIO10.0016
BNL8. 17
PIO20.0075
PIO20.0042
 АВВНННВВННАННАААВАНАВНАННАНАНАНННВАВННВВНАННВННННННАНАВНААНААННАВАНВНАВААААААНННАНАН ННННННВНННАНВАННВВНННВАННАААНААВННННН-НННННАННАННАВНААННААННАААНВААВНАВННАНАНАННННАН НВВНННВНАНАНВАААВАННННАНННААНННВВННВННВННАННВННННННАНАННААННАНААВААВНАВАААААНАННАНА-НННАННВНАННВННННВНННННННВНАНАННННВА-ВААНАНННННВАНННнННВННВНАНАНАНАНННАВНННАННВНННВАВ НВВННHBBHHAHHAA--AHABHAHHAHAHAHHHBABHHBBHAHABHHHBHHAHABHAAHAAHHABAHBHABBAAAAAHHHAHAH ННВННВВВНHABBHAHBAAHBHAABHHHHAAHHBAHBHHHHHHHHHBAHHAAHABHHAHAHHHAHHHHAABBHAAAAHHHHBAH АВВНННВНАНАНВАААВАНАННАНННААНННВВННВННВНААННВННВНННАААННААННАНААВААВНАВАААААНААНАНАН ННВННАНАННАНННВААНННННННАННАНААННВВНННАННВВВНАННВНННААААННВННН- НВ ННВ ВААННВНВВАННHHBHHHAHHBHHHHAHHHHHBAHHBHBHABBABBBHHHAHHHHAHAHBHHHA-AHAHBAHAAHAHABHHAHA ННАНААННВНАННННАААННННННННААН-ННННАНВННННВВВННННВААННАННАНВАВНВАНННАНВАНААНАНННННННА
 НАВВНВНВВАННННВНННАНАВННННАВННННВАННВНВНАВВАВВВНННАННВНАНАВВНН-ННАНААНАВНННАНАВНВАНА HНАНААННВНАННННААА----ННННААНАННННАНВННННВВВННННВААННАННАНВАВНВАНННАНВАНААНАНННННННА ННАНААННВНАННННАААННННННННААНАННННАНВННННВВВННННВААННАННАНВАВНВАНННАНВАНААНАНННННННА АНННАВННВННННАВВ-ВВНА-ВННННННВНВНВНАВНВАААННННННННАНАНВBHH-HH---HHHBBH-АНВНАВНHHHHHH

 АВННАВННВНАННАВВННВВАНВНННВННВНВНВНАВНВААААНННННННАНАНВВННННВН-ННННВВННАНВНАВНВННАНН HНHHAAAHBBHAHHHHBHHHBHHHBHHHHHHHAHHBAHHAAHBHBBAAAHBBAAAHBAAAAA-AHBHBAHHBBBHAHABHBAHB
 НААННАН-В-АНВННААННННННННВААААННННА-ВННННВВВНВВНВААННАННАНВАВНВ-НННАНВННААНАНННННАНА
 ВНАНВBBBBBBHBHHHBBHAAHBHBAHHHABBHHHHABHHBHBABAHBHHHBBBHBHHAHAA-BHHBB-ВHBHAHABABAHHHH ABHAHHHAAHBHBBBHAHBHHHBABHHBHAHHAHBHHHHHHBBHHBAAHHHHBAHHAAHBAABHHBHHBHAHAAHAAAHHHHBA ННААННННВНВНВНВAAHB----BHHABBHHAHH-ABAAA-A----AHHHHAHBBBH-BHHABAA-HHABAHAHBHHHAHBABA HHHA-------AHH-HH-H---HHHHHHAAHH--BABHHHAH--AHHHBH-HHHBBH-BHAAHH--HAH-ABBAHHA-HABB-B ВАНВННАВАВННВВНВНАВННАНВНААНВВННВНААНВАВАН-ВВНВНВААНВНАНВВАННН-НАНННВНВННННВАВНННННА ВАНВВНВВНННВВНННВВНННВННВАААНННННВННААННВН-АНАНВНННАНВННВАНВААНННННВНВННВНННННННННАА --НВААНН-- ННННАААНА - ННННААНАН--ВААВННААА-ВАННВВАНННАННАВВАВВ-АВННА-В-ННАНАННННВННА HHHBAAAHBBAHHHHBBHHHBHHHBHHHHHHHAHHBAHHAAHB-BHHAAHHBAAAHBAAAAAHAHBHBAHHBBBHAHHBHBAHB ВАННННАНАВВНВВНВВАН--AHBHAHHBBHHBHAA-BA-AH-BBHBHBAAHBHAABHAHH--H-HHHBHBHHBHBABHHHHHA

BNL12. 06
UMC157
UMC161
UMC23
NPI234
BNL5. 62

HHHHHAHBAHAHHHHBHAHHHHAHHHHHHHHAAABHBHHBHHHHAABBHHHAABBHBHHHBHHHBHHHHHHBHAHBBHHABHHBH ННВНННННААНАНННННАВНАНВННАННННАААНВНААННАВНААНВННАНАААВННННННННВАНВНВНАВНННАВАВАВН--А ВНАВВВН-АНННННААНАВННВНННАВАННННАААННHHBHBBAABHHABBAAAHBHBHHHAH-HHAHHBAAABAHAHHHBHBBH ВННВНННВНВАННВНННННВАВВВНАННВВВНН--АВАННАННААВННННВНАВНАННННННННАВНВАНННАННАННАННАННА HHHHHHH-AAHAHHHBHAHHAHHHH-HHAHHAAABHAHHHABBHAABHHAHAAAHHHHH-HHHHBHH-BBHB-AHHBAAABHHBA НАВННННВНВАНАННННАНВННННВНАВНААААВВННАННВННААНВННННАНВНАВННВВАНННННАНАВВННАВНННВНАННH

BNL15. 18
BNL8. 29
UMC128
UMC58C UMC33
UMC164
NPI1429
UMC61 UMC78 NPI287 (1) NPI287(2)
UMC5
UMC5 3
UMC88
AGP2
UMC34
UMC98
UMC135
UMC131
BNL6. 20
BNL1. 297
NPI565
BNL15. 20
UMC60
BNL8. 15
UMC32
UMC39
UMC175
UMC121
UMC26
BNL 7.O8 (1)
BNL7.03(2)
UMC16C3
BNL5. 37
UMC50
BNL15. 27
BNL7. 65
UMC158
UMC31
UMC15
PIO10. 25
BNL5. 46
BNL15.07

ВААВНННВАНАН-АННАНАВАВНВННААВВВНННВАВАННННH-HBHHHHHABBBAHHBHBBHBABHBAA-HAAHAAAHHHAH-H ВАННВАВНААНВННННННННАННВНННАВВННАННАНАННВАВВАННННННАВВННННННАВНВАННВААНННАННАААВННННН ВААВНННВНВААНВННHHHBABBBHHAABBBHHHHAВАНHAHHHHBHHHHBHABHAHHHHBHHHABHBAHHHAHHAHHAHHAHHH
 ВННВННHBHBAHHBHHHHHBABBBHAHHBBBHHBBABAHHAHHAABHHHHBHABHAHHHHHHA-АВHBAHHHAHHAHHAHHAHHA ВНВВНВНВНВНННHAHBABHHAHBHAHAHBHHAAABHAHHABABHHHHABBHHHHAABHHHAHHHHHHBAAABAHBAHABHBBA
 -НААВАН-HBAAAA-HHHHBBAHHA-BAAHAHHHAAAAAAHHHHABHHHHAHBHABAA-BAAB-HHHHHHHBAHAAHHHA-BBHH НВАНВАНВНВННААНННННВВАННААВААНАВВАНААНААННННАВВНВНННВНАННАНННАНННВНВНнНннННАНнНАВВВВВ ННААВАНВНВААААНННННВВАННАНВААНАНННААААААННННАВННННАНВНАВААНВНАВ-НННВНННВННААНННАВВВ-HHHBHHA-BHHHBBHAHHBABHHABAHHHAHABHHAABABHBHHHAAHAAABHHHBHHBAABH-BBBHBAHHAHHBHHHABHHВВНАНАВВВАААНВВАннHHHHAHHABBBHAAABAAHHHHHHHHHHHHHBHBBAHBHHABAHHBAHABBHHBHBAHHHAABHBHA HBABBABBHBBHBHHHHHHHH-BHHHBAAAHHBAABAHAHHABHBBBHHHHHBHBHHAHHHAHHHHBBAAHHAHHAHABAAHBBB
 ВВНАНАВВВНААНННАНННННАННАВНВНАА-ВНАННННВННННННННВНВВАНВНННВАННВННАВВННВНВННННААВНВНЕ ВННАВАННННААААННHHHBBHHHHHBAHHAHHBAAHHAAHHHHABHHHHABBHABAHHBAHHBHHHBBHHBHHAAHHHABBBHH ВВНАНАВВВАААНВВАНННHHHAHHABBBHAH-B-АНHHHBHHAHHHHHBHBBHABHHHBAHBBAHABBHHBHBHAHHA-BHBBH BВНАНAВНВНААНHHHHHHHHHHHHABHBHAAABHAHHHABHHHHHHHHBHBBAHBHHHBAHHBHHABBHHBHBAHHHAABBBHF АННАНАВНВНААННННВВННННННАВНННАААВАНННААННННАНННВНАВВАНВНННВАННВННАВВННВНВАННННавВВНН ВННАНнНВВН--HBBAHHAHHHAHBHBBBHAHAB-HHHHBHHHHBHHHHHBBAHHHHBAHAHHBHHABHAHBBBHHHBAAHHBHA ВАВНННВВНВВНННАННАНАНААВНАНАНВННАААВНННВАНАНННААНВВННННААВНННАННВАНННВНАННАННААНВНАА BBHAHHBBBHAHHH--------АHHABHBHAAABAAHHHHBHHHHHHHHBHBBAHBHHHBAHHBHHABBBHBHBAHHHAABHBHH ВАВВННHBHBBHHHAHHAHAHAABHAHAHBHHAAABHHHBAHABHHAAHBBHHHHAABHHHABHBAHHBAAHHAHBAAHBHBBA ВАВВНВНВНВВНВНАННАНАНААВНАНАНВНАААНВНАННАНАВННАНАВВННННАНВНННАНННнHHНВААННАНВанНВНВВА
 -НАВАННННВАААААААВННАНННВВАННАААНВННАНННАНННВНАНВВНННВННАННАААНВННАААВАНВАНВАНННАНННА -HBBHHH-HBBHHHAHHAHA--ABHAHAHBHHAAABH-HBAHAHHHAAHBBHHHHAABHHHA-BBHHHBBHHHHAHBHAHBH--A ВАВНННВВНВВНННАН-АНАНААВНАНАНВННАА-ВНННВАНАНННААНВВННННААНННННННВАНННВНАННАННААНВНВВА ВНАВВBHHHBAAAAAAABBHABHHBBAAHAAAHBHHAHHHAHHHBHABBBHHHBHHAHHAAAHBHHAAHBAAHAABAABHABAHA ВННВВВННАННАННААВАВННННННАНАННННАААННННННВННАВННАВВНААНННВНННАНАННАВВВАAABABAHHA-H--H АННВННН-НВННАНАНННННАВНАНАННВННННВВАНААААННААВВАНАНААННАННН-ННА-АННВА-ННАННАННАН-А--А АВННHHH-ВНААНHHHHAABBHBAABHHABBHBHHBHBHABHBAHHHBBAHHHBBAHHH-HHH-BHHAB-BBHHHHHHHA-H--H ВАВНHHBBHBBHHHAHHAAAHAABHAHAHBHHAAABHHHBAHAHHHAAHBBAHHHAAHHHHHBABAHHHBHAHHAHHAABBHBBA ВНВВВВНВНННАННАНВАВННАНВНАНАНВННАА-ННАНННВАВНВННАВВНАААННВАННАННННАННВАААВААНННАВНВВ ВНАВВВННННННАНААНАВННВНННННАННННННАННННВНВВААНАНАВВАААНВНВНННАНАННААНВАААНАНАНВНВНВВН НАВАННННАВАНАННВНААНННННВВВВННААНВННААВНВННННАВНАННН-ННАННВНАВННАННННННАНАААВВАННВ--В ААНВАНННННННААНВНВННВНАНАНВВВНАНННВНААНННВННВНВНААААНВВНАНННВНВВАНВВННнHHAAHHBAAHHHHB ААНВННHHHHHAAAHHHBHHBHAHAHBBBHAHHHBHAAHHHHHHHHBHAAAAHBBHAHHHBHHBHHBBHHHHBAAHHBAAA-HHB ВАВАННHHABAHABBBHBBBBHHAHBHBHBHHHBHHAHAHBAHHHABBAHHHHHHHHHAHAHHHAHHHABHAHAAHHHAHHHBHB ААННННН-ННННААНННВНАВНА-АНВВННААННА-ААНВ-НННННВВНАААН--HAHHHBHA-HHBBH-HHBAAHHBAA-HH-B АНННННННННННААНННВНАВНАНННВВННААНННАААНВННННННВННАААНВВНАНННВННВННВВННННВААННВААННННВ НАВАННННАВАНАВВВНВНВВННАНВАВНВНННВННАНННВАНННАВВАНВННННАННННАННААННННННАНААНННАНННВНВ АВНВНАНВНВННАННННВНАНННВВНВННННААВААНАНВНАНННВВННHAHAHBBHBHHBBHBAHBBHHBHHAABHHHHBAHHA

NPI292
NPL203 UMC90
JC162
UMC166
UMC104
BNL7. 43
BNL10.06
BNL6. 25
BNL7. 71
BNL5. 02
UMC51
UMC67
UMC68
BNL8. 33
PIO10. 16
UMC70
NPL280
UMC21
BNL3. 03
UMC62
UMC85
BNL15. 40
DEK326
BNL13. 24
BNL14.07
BNL8. 37
BNL16.06
UMC110
UMC116
UMC80
BNL8. 39
BNL15.21
BNL9. 08
NPI268
UMC89
NPI220
BNL9.44 UMC103 BNL8. 26
BNL14. 28
CIC9S
UMC114

ААН--ВВНАННHAAHBHBHHBHAHAHBB-AAHHHB----HH-HHBHBHAAAAHBBHAHHBBHHHAHBBHHHHHAAHHBAAHHHBB АННВНННННВННААНННВНАВНАВН-ВВННАААНААААНВННННННВННАААНВВНАНННВВННАНВВННННВААННВНННН--В АНВВНВААННАНННННННВННННАВНННАВАВВААВННВННААНААНАНННВНВВННААННННАНААНАНАААВААНННВАВНВА АННВАВАННАНННВННННВНННВАВАНВННННННАВНННАНАННННННАНАВАВНННАННАНННННВННHHHAHAAHHBHABHBA AHHBABAHHAHBHBHHHHBHHHBABHHBHHHHHHHBHHHAHAH-HHHHAHABABHH-АHHAHHBHHBHHHHHAHAAHHHHABHBA ВННВННHABAHHAAHBBBBHABAHBHHHBBHAAHHHAHAHBAHAHABBAHAHABAHHHHBAAHBHHHHBBHHBHAHAAHBHHBBA ААНВАВАНВАНВНВНВННВННВВАВААВНННННВАВНННАНАВНННАНННАВАВАНВНННАНННААВНВНННАНААННВНАВНВА ААНВАВАНВАНВНВНВННВННВВАВААВННHHHBABHHHAHABHHHAHHHABABAHBHHHAHH-AABHBHHHAHAAHHBHABHBA АНВВНВААНВАННННННнНННННАВНАНАВАВАААВНАНННАНВННАннНнВНВНАНННАННННННАНАНААНВННАННВАНВВН ААНВАВАНВАНВНВНВННВВНВВАВААВНННННВАВННВАНАВНННАНННАВАВАНННННАНННАНВНВНННАНААННВНАННВА АННВАВА-НАНВНВННННВНННВАВННВНННННННВНННАНАННННННАНАВАВННВАННАННВННВНННННАНААННННАВНВА НААВНННВВНВННВВВАНВНАВВААННВВАНААВАВААННННННВАВНАВАААВАВННВАНАННННАННААНАНАНАВННВННВН НВВВВВВНАВННВНВАНАВВАВНННАННВНААНВВННАВААННААНВННАВААННННННВННАНАНВННВАВНННАВНННВНВВА ВННВНHHABHBHAABBHHHBABHHAHHBBAHAAB-HAAHHHABHHAAHABAHABAHBHBHAABBHHAHHAHHAHAHABHHHHHBA
 ВВН-ННА - ВНННВННАННВАВВНННА - В ВАНАНВАНАВ -АНВННН-ААВВННАНННВВВВBHAHHHBBBHHABBHHABHBBHBHAHHHHHHHBBBBBBA - - HHBHBBABBHHAABHH-HHAH НННАВННВННВННАВВНННННННННВВННННВНАААНВННАААННВВННАВАААВНННННВВННННАНННННААААННННАНВВА ВНHHBHHHBABHHHBAHHHAHBBHBBHBABABBAAABAHHBHABHAHHHHBBHBBHAHHHBHHBHHABABBHHAHAHHHAAHHBH


 ВНННННАННАААВВАНВНННННННННАНННННВНННААНННАНАВНННАННВННАНННННННАААНАНАВВНВАНННННВННВНВ BHHBHAHAHBAAHBHHHHBHHHHHHHAHAHAHBAAHHAHHAAAABBBBHHHHBHABHHHAHHHHHHAAHBBHBHHHAHHBAAHHH ВННННННАННААНВННННВННВНВННАНАНАНВАНННАННААААВВНHHHHHBHAHHHHHHHHHHHAAHBBHBHHHABABAABHF ВННHHHHABHAAHB-НHHBHHBHHHHAHAHAHBAHHHAHHAAAABBBBHHHHBHABHHHAHHHHHHAAHBBHBHHHABHBAAHHH ВННННННАВНААНВННННВННВННННАНАНАНВАНННАННААААВВВВННННВНАВНННАНННАННААНВВНВНННАВНВААННН ННАНННН-ВНАННВВННННВНАННАН-ВАНВНВАНННВНВААААННВВНВНВНННВНННННННВВНАННВННВ - - АВННААН-Н ВННННННАННАА - ВННННВННВНВННАНАНАНВААННАННААААВВННННННВНАНННHHHHHHHHAAHBBHBHHHABABAABHE
 ННАНННH-ВВНННВНННННННАННАНАНАНВНВАНННВНА-Н-АНВВВНВНВВННВНВННHHHBBAAAHBHHHHHHABHHAHHBH
 ВНННННААННААНВННВНВНННННННАННННВВНАНААНННАНАВВНННННВВНАНННННННАНННАААВВНВАНННВНВАНВНН НННАНННВВННАНННННААВННВААВННАВВНВНАВННВНВНВАНННВВАВННВНАНННННННАННВАНАНВННННННННННАН АВААНАНАННАНННАНВ--ВНННААНHHHHBHAAAHHHBAHABAHBAHBHBHAHHHHBHHBHH-ВHHHBHHBHAHAHBHABBBBH -ВНАНАНВВАААНАННВААВННВААНННННВНННАННВВАВАВАННННВАВННВАНАНННННННВНАННАВВНННАНННАВН---
 НННАНННВВННННННННААВНАВААВННАВВНВВВВННВНВНВАНННВВАНННВНАНННННННАННВАНАНВННННННННННАНН ВНВВНААВВННААНННАНАВАВВААВННННВНВВВАННВННАВАНВНВВАНННННВНАННВНННННВАНННВАНННВНННННННА -ВНАНАНВВАААНАННВААВННВААННHHHBHHHAHHBBABABAHHHHBABHHBAHAHHHHHHABHA-HABBHHH-HHHABH-HH АНННАВАВННВВНН-НАВННАВАНВВВНННВВНА-НВАНННАННВВНHHHAHAAHAHHHBHHHBHAH-НHAHABHAHAHBBBBHH ВАННННННААВВННАНННВВНВНВАНННВВВНВАНННВАВНВВНВНАНАННHHHBBBBHBHHHHHHBHHHHBBHHAABHBBHHAB НАААНВННАНВНВНВВАВВВАВАВННВННВННННВННАННАННВННАНННАНАННННАНВНННВВННВНННВАНННАНННВНННВ

UMC20
PIO10. 5
UMC81
UMC153
PIO10. 33
UMC64
NPI 3034
NPI 232
BNL10. 17
BNL5. 71
BNL3. 06
NPI560
PURPLE
UMC11
NPI457
NPI250
PHI10. 17
PIO10. 0016
BNL8. 17
PIO20. 0075
PIO20.0042

НАААНВННАНВНВНВНАВВВАВАВННВННВНННН-ННАННАВННННАНННАНАННННАНВНННВВННВНННВННННАНННВНННВ -АН-НHH-AABB-HAHHHBBBBBHA-HHBBBBHAHBHHABHBB-BHAHHHHHHHBBBBBBABHABHBHBBABBHHAABHHBHHAB HAAAHBHHAHBHBHBBABBBABABAHBHHBHHHHHHHAHHABHBHHAHHHAHAHHHHAHBHHHBBHHBHHHBHHHHAHHHBHHHB
 ВННHHHA-ВHHHB--АНHBAH-HHHAHHBAHAHBAHABAHHBAB--HAAAAHBHHBAAHAABHBHHHHBAHABHHHHHHABAHHA ВНННННАВВНННВНННННВАВВНННАННВАНАНВАНАВАНВВАВНАНАААА - ВНВВА-Н-H-ННННННВННАНННВНННА-АННА В-ННВНАВВНННВННАННВАВВНННАННВАНАНВАНАВАННВАВНАНААААНВННВААНААВННННННВАНАНННВНННАВАННА ВНННННА-ВНННВНHAHHBABBHHHAHHBAHAHBAHABAHHBABHAHAAAAHHHHBHAHAABHHHHHHBAHAAHHBHHHABAHHA АННHH-AABBAHHABAAHAHHAHBHAHHHHBHABHABBABBHHHBHBHAHHABAHHHBBAHHBBAHHAHHBAHAHBAAHHAHBBA
 -АААННН--НВНВНВВАВВВАВАВАНВННВВНВННННАНННВНВННАНННАНААННВННВНННВВВВВНННВННННАННВВНННВ ВНВАВААНВАВНННВАНННАННВНВВННАНАВВАААВАННВННВННННАНВВНВВННННВВНАНННАВАВВННАНАНННААННВ ВНА-НВН-ННВНН-НАН-ВААВННВННВНВАВВАА-ВАННВНАВНАН-АНВВННВНАВННВННВННАВАВВННАНННННААННВН
 AAH-BBB-HB-HHH-HHAAH--HBHAAAAH-HBHHBHAHBABAHHAHAHBBAHHHHAAH-BHH-HHHHHBHHBBAHAAHBHHBBH
 В-НВНННАВАН-АА-НВНВННВАНВНННВВНААНННАНАНВАНАНННВАНАНАВАННННВААНВННННВВННВВАНААНВ-Н-ーA ВННАВААВВНВНННВВН-Н-- ВННВНННААВНАААННННААНВНВННАНВНАВВННН-НВНН-ННАННВВННААА-ННААНН--BAA-AB--------BABBBA-AB-----B--HH-HHAHHAHHBHHAHHAAHAAHHBHABHHHBBBHBHHBBAHHHAB-H-H---AHHHHBAABBAHHAHAAHHHH-HBHHHHHABHABAABBAHBBHHBABHAHHABHBHHHHAHHH-AHHAHHBAAAHBAAHHAHBBA ВНН---НАВА-НАА-ВНВННАВАНВВВНВВНААНАНАААННАВАААВНАНАНАВАННННВАAHB-HH-BB-HHHA-AHHH-----

APPENDIX 4. TEST FOR SEGREGATION RATIO OF RFLP MARKERS


| MARKER | \#A | \#H | \#B | \#- | $F(A)$ | $F(B)$ | $x^{2}$ | P |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 51 NPL203 | 30 | 101 | 32 | 6 | 0.49 | 0.51 | 9.33 | 0.0094 |
| 52 UMC90 | 48 | 89 | 32 | 0 | 0.55 | 0.45 | 3.43 | 0.1803 |
| 53 JC162 | 45 | 98 | 26 | 0 | 0.56 | 0.44 | 8.30 | 0.0158 |
| 54 UMC166 | 42 | 96 | 26 | 5 | 0.55 | 0.45 | 7.90 | 0.0192 |
| 55 UMC104 | 40 | 78 | 48 | 3 | 0.48 | 0.52 | 1.25 | 0.5343 |
| 56 BNL 7.43 | 49 | 87 | 31 | 2 | 0.55 | 0.45 | 4.15 | 0.1253 |
| 57 BNL 10.06 | 49 | 87 | 32 | 1 | 0.55 | 0.45 | 3.65 | 0.1608 |
| 58 BNL6. 25 | 40 | 96 | 32 | 1 | 0.52 | 0.48 | 4.19 | 0.1230 |
| 59 BNL7.71 | 46 | 91 | 32 | 0 | 0.54 | 0.46 | 3.19 | 0.2034 |
| 60 BNL5. 02 | 41 | 99 | 27 | 2 | 0.54 | 0.46 | 8.06 | 0.0178 |
| 61 UMC51 | 44 | 89 | 36 | 0 | 0.52 | 0.48 | 1.14 | 0.5654 |
| 62 UMC67 | 42 | 76 | 51 | 0 | 0.47 | 0.53 | 2.88 | 0.2367 |
| 63 UMC68 | 46 | 83 | 39 | 1 | 0.52 | 0.48 | 0.61 | 0.7382 |
| 64 BNL8. 33 | 33 | 103 | 33 | 0 | 0.50 | 0.50 | 7.67 | 0.0216 |
| 65 PIO10. 16 | 35 | 79 | 37 | 18 | 0.49 | 0.51 | 0.38 | 0.8263 |
| 66 UMC70 | 30 | 81 | 50 | 8 | 0.44 | 0.56 | 5.00 | 0.0821 |
| 67 NPL280 | 33 | 98 | 38 | 0 | 0.49 | 0.51 | 4.30 | 0.1166 |
| 68 UMC21 | 32 | 85 | 51 | 1 | 0.44 | 0.56 | 4.32 | 0.1152 |
| 69 BNL3. 03 | 32 | 77 | 47 | 13 | 0.45 | 0.55 | 2.91 | 0.2334 |
| 70 UMC62 | 37 | 93 | 39 | 0 | 0.49 | 0.51 | 1.56 | 0.4578 |
| 71 UMC85 | 28 | 96 | 44 | 1 | 0.45 | 0.55 | 6.48 | 0.0392 |
| 72 BNL15.40 | 30 | 102 | 37 | 0 | 0.48 | 0.52 | 7.42 | 0.0244 |
| 73 DEK326 | 33 | 101 | 34 | 1 | 0.50 | 0.50 | 6.89 | 0.0319 |
| 74 BNL13.24 | 31 | 103 | 35 | 0 | 0.49 | 0.51 | 7.86 | 0.0196 |
| 75 BNL14.07 | 29 | 97 | 41 | 2 | 0.46 | 0.54 | 6.06 | 0.0483 |
| 76 BNL8. 37 | 31 | 98 | 39 | 1 | 0.48 | 0.52 | 5.43 | 0.0663 |
| 77 BNLI6.06 | 31 | 88 | 39 | 11 | 0.47 | 0.53 | 3.08 | 0.2149 |
| 78 UMC110 | 30 | 103 | 35 | 1 | 0.49 | 0.51 | 8.89 | 0.0117 |
| 79 UMC116 | 31 | 99 | 36 | 3 | 0.48 | 0.52 | 6.82 | 0.0330 |
| 80 UMC80 | 31 | 94 | 39 | 5 | 0.48 | 0.52 | 4.29 | 0.1169 |
| 81 BNL8. 39 | 37 | 89 | 40 | 3 | 0.49 | 0.51 | 1.12 | 0.5700 |
| 82 BNL15.2 | 7 | 33 | 100 | 35 | 0.49 | 0.51 | 6.14 | 0.0464 |
| 83 BNL9.08 | 45 | 91 | 33 | 0 | 0.54 | 0.46 | 2.57 | 0.2772 |
| 84 NPI268 | 45 | 91 | 29 | 4 | 0.55 | 0.45 | 4.67 | 0.0966 |
| 85 UMC89 | 49 | 84 | 31 | 5 | 0.55 | 0.45 | 4.05 | 0.1321 |
| 86 NPI220 | 29 | 105 | 28 | 7 | 0.50 | 0.50 | 14.75 | 0.0006 |
| 87 BNL9.44 | 42 | 90 | 35 | 2 | 0.52 | 0.48 | 1.60 | 0.4504 |
| 88 UMC103 | 40 | 90 | 39 | 0 | 0.50 | 0.50 | 0.60 | 0.7395 |
| 89 BNL8. 26 | 54 | 79 | 32 | 4 | 0.57 | 0.43 | 6.29 | 0.0431 |
| 90 BNL14.28 | 36 | 95 | 34 | 4 | 0.51 | 0.49 | 3.54 | 0.1703 |
| 91 CI | 33 | 90 | 45 | 1 | 0.46 | 0.54 | 2.57 | 0.2765 |
| 92 UMC114 | 37 | 100 | 31 | 1 | 0.52 | 0.48 | 6.52 | 0.0383 |
| 93 UMC20 | 37 | 103 | 28 | 1 | 0.53 | 0.47 | 9.56 | 0.0084 |
| 94 Prol0.0005 | 33 | 75 | 54 | 7 | 0.44 | 0.56 | 6.13 | 0.0467 |
| 95 UMC81 | 38 | 96 | 31 | 4 | 0.52 | 0.48 | 4.69 | 0.0956 |
| 96 UMC153 | 37 | 102 | 30 | 0 | 0.52 | 0.48 | 7.42 | 0.0244 |
| 97 PIO10.33 | 35 | 87 | 34 | 13 | 0.50 | 0.50 | 2.09 | 0.3517 |
| 98 UMC64 | 33 | 94 | 36 | 6 | 0.49 | 0.51 | 3.93 | 0.1404 |
| 99 NPI303U | 37 | 95 | 36 | 1 | 0.50 | 0.50 | 2.89 | 0.2354 |
| 100 NPI232 | 39 | 90 | 38 | 2 | 0.50 | 0.50 | 1.02 | 0.5994 |


| MARKER | \#A | \#H | \#B | \#- | F(A) | F(B) | $\mathrm{x}^{2}$ | P |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 101 BNL10.17 | 49 | 77 | 41 | 2 | 0.52 | 0.48 | 1.77 | 0.4119 |
| 102 BNL5.71 | 29 | 97 | 31 | 12 | 0.49 | 0.51 | 8.31 | 0.0157 |
| 103 BNL3.06 | 35 | 87 | 40 | 7 | 0.48 | 0.52 | 1.35 | 0.5100 |
| 104 NPI560 | 33 | 93 | 43 | 0 | 0.47 | 0.53 | 2.71 | 0.2586 |
| 105 Pl1 | 32 | 75 | 54 | 8 | 0.43 | 0.57 | 6.94 | 0.0310 |
| 106 UMC11 | 43 | 89 | 30 | 7 | 0.54 | 0.46 | 3.84 | 0.1467 |
| 107 NPI457 | 41 | 71 | 36 | 21 | 0.52 | 0.48 | 0.58 | 0.7479 |
| 108 NPI250 | 26 | 72 | 23 | 48 | 0.51 | 0.49 | 4.15 | 0.1255 |
| 109 PHI10.17 | 39 | 78 | 44 | 8 | 0.48 | 0.52 | 0.54 | 0.7649 |
| 110 PIO10.0016 | 32 | 89 | 38 | 10 | 0.48 | 0.52 | 2.71 | 0.2576 |
| 111 BNL8. 17 | 40 | 61 | 31 | 37 | 0.53 | 0.47 | 1.98 | 0.3707 |
| 112 PIO20.0075 | 47 | 81 | 38 | 3 | 0.53 | 0.47 | 1.02 | 0.5992 |
| 113 PIO20.0042 | 41 | 66 | 40 | 22 | 0.50 | 0.50 | 1.54 | 0.4629 |

APPENDIX 5. MEANS FOR EXPERIMENTS 89102,89302 and 90102 F3 LINES FROM POPULATION B52XMOI7, PARENTAL LINES AND HYBRID BETWEEN THO PARENTAL LINES*






P $\overrightarrow{A l}$
Pedigree 852XM017-F3-001 352XM017-F3-00 BS2XMo17-F3-003 352XM017-F3-004 52XM017-F3-005 852XMO17-F3-006 852XMo17-F3-007 352XM017-F3-008 $852 \times M 017-\mathrm{F3}-010$ 352XMo17-F3-011 352XMo17-F3-012 B52XMo17-F3-013
B52XM017-F3-014 352×4017-F3-015 B52XMo17-F3-016 352XM017-F3-017 52XM017-F3-019 52XM017-F3-019 $852 X M 017-F 3-020$ 52XM017-F3-022 52XMo17-F3-023 552×M017-F3-024 52XM017-F3-025 $352 \times M 017-F 3-026$
$52 \times M 017-F 3-027$ S5XM017-F3-028 5 2XMo17-F3-029 52XM017-F3-030 2XM017-F3-03
2XMo17-F3-032 52×M017-F3-033 52XM017-F3-034 52XM017-F3-035 $52 \times M 017-F 3-037$ 52XMo17-F3-038 $352 \times M 017-F 3-040$ 52XMo17-F3-041 $352 \times M 017-F 3-042$
35 XM017-F3-043 52XMo17-F3-043 852XM017-F3-045 352xM017-F3-046 S2xMo17-F3-047 $352 \times M 017-F 3-048$
$352 \times M 017-F 3-049$ 52XM017-F3-050 52XM017-F3-05 852XM017-F3-052 52XM017-F3-053 52XM017-F3-05 52XM017-F3-055 $852 \times M 017-F 3-056$
$852 \times M 017-F 3-057$ 352XMo17-F3-058 352XM017-F3-059 52xM017-F3-061 $52 \times M 017-F 3-062$ 52XM017-F3-064 $352 \times M 017-F 3-064$
$352 \times M 017-F 3-065$ 52XMo17-F3-066 52xM017-F3-067 52XM017-F3-068 $352 \times M 017-F 3-069$
$352 \times M 017-F 3-070$ $52 \times M 017-F 3-079$ 52XM017-F3-072 352XM017-F3-074 $352 \times M 017-F 3-074$
$352 \times M 017-F 3-075$ 52×M017-F3-076 352XMo17-F3-077

$262.5 \quad 251.6$



SL Ong
Ong 45


[^6]






1143.5 11411
1220

[^7]* Adjusted (Adj.) and unadjusted (Unadj.) means were listed for traits when efficiency of lattice design was larger than 105\% Unadjusted means were listed for traits when efficiency of latrice design was lower than $105 \%$.

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[^8]Env. 1 ECB


Env. 3 ECB



Env. 2
ECB

Combined Data


Figure 7A. DISTRIBUTION OF $\mathrm{F}_{2: 3}$ LINE MEANS FOR 2ECB TUNNELLING


N

Figure 7B. DISTRIBUTION OF $F_{2: 3}$ LINE MEANS FOR MORPHOLOGICAL TRAITS IN HILL PLOTS

Env. 1


Env. 2



Figure 7C. DISTRIBUTION OF $\mathrm{F}_{2,3}$ LINE MEANS FOR MORPHOLOGICAL TRAITS IN SINGLE ROW RLOTS


Env. 2


Combined Data


Figure 7C (Continued). DISTRIBUTION OF F ${ }_{2: 3}$ LINE MEANS FOR MORPHOLOGICAL traits in single row plots




Figure 7C (Continued). DISTRIBUTION OF $F_{2: 3}$ LINE MEANS FOR MORPHOLOGICAL
TRAITS IN SINGLE ROW PLOTS

Env. 1


Env. 2


Combined Data


Figure 7C (Continued). DISTRIBUTION OF $F_{2: 3}$ LINE MEANS FOR MORPHOLOGICAL traits in single row plots

```
GY (g) -
```

Env. 1


Env. 2


Combined Data


Figure 7D. DISTRIBUTION OF $F_{2: 3}$ LINE MEANS FOR YIELD COMPONENTS IN SINGLE ROW PLOTS

EN (ear) -

Env. 1


Env. 2



Figure 7D (Continued).

KW (g)

Env. 1


Env. 2


Combined Data


Figure 7D (Continued).

EL (cm)

Env. 1


Env. 2



Figure 7D (Continued).

KR (row)


Env. 2



Figure 7D (Continued).

EW (cm) -


Env. 2


Combined Data


Figure 7D (Continued).

```
CN (cm) -
```





Figure 7D (Continued).

KD (cm) -

Env. 1


Env. 2



Figure 7D (Continued).

## APPENDIX 8. TABLE OF ANALYSIS OF VARIANCE

RECTANGULAR LATTICE DESIGN

| Source | d | MS | EMS |
| :---: | :---: | :---: | :---: |
| Replications | ( $\mathrm{r}-1$ ) |  |  |
| Blocks (Adj.) | rk |  |  |
| Entriea (Unadj.) | $k^{2}+\mathrm{k}-1$ |  |  |
| Intrablock Error | $(r-1)\left(k^{2}-1\right)-k$ |  |  |
| Genotype (Adj.) | $\mathrm{k}^{2}+\mathrm{k}-1$ | $\mathrm{M}_{2}{ }^{\prime}$ | $\sigma^{2}+\mathrm{r} \sigma_{8}{ }^{2}$ |
| $F_{3}$ Lines | 1-1 | $M_{2}$ | $\sigma^{2}+r \sigma_{F 3}{ }^{2}$ |
| Residuals | $k(k+1)-1$ |  |  |
| Effective Error | $(\mathrm{r}-1)\left(\mathrm{k}^{2}-1\right)-\mathrm{k}$ | M ${ }_{1}$ | $\sigma^{2}$ |
| Total | $r k^{2}+r k-1$ |  |  |

r=number of replications in each environment
k=number of plots in each block
COMPLETE RANDOM BLOCK DESIGN FOR INDIVIDUAL ENVIRONMENTS

| Source | d | MS | EMS |
| :---: | :---: | :---: | :---: |
| Replications | ( $\mathrm{r}-1$ ) |  |  |
| Entries | $\mathrm{g}-1$ | $\mathrm{M}_{2}{ }^{\text { }}$ | $\sigma^{2}+\mathrm{r} \sigma_{\mathrm{g}}{ }^{2}$ |
| $F_{3}$ Lines | 1-1 | $\mathrm{M}_{2}$ | $\sigma^{2}+\mathrm{r} \sigma_{\mathrm{F} 3}{ }^{2}$ |
| Residuals | $g-1$ |  |  |
| Error | $(g-1)(r-1)$ | $M_{1}$ | $\sigma^{2}$ |
| Total | rg-1 |  |  |
| $\begin{gathered} \text { g=number of } \\ \text { this study } \end{gathered}$ | rcluded in |  | ber of |

COMPLETE RANDOM BLOCK DESIGN FOR MULTIPLE ENVIRONMENTS

| Source | d | MS | EMS |
| :---: | :---: | :---: | :---: |
| Environments (Env.) | (e-1) |  |  |
| Replications/Env. | $e(r-1)$ |  |  |
| Entries | g-1 | $\mathrm{M}_{2}{ }^{\prime}$ | $\sigma^{2}+r \sigma^{2}{ }_{\text {grc }}+r e \sigma_{z}^{2}$ |
| $F_{3}$ Lines | 1-1 | $\mathrm{M}_{2}$ | $\sigma^{2}+r \sigma_{\mathrm{F} 3 \mathrm{cc}}+r e \sigma_{\mathrm{F} 3}{ }^{2}$ |
| Residuals | $\mathrm{g}-1$ |  |  |
| Entries x Env. | $(\mathrm{e}-1)(\mathrm{g}-1)$ | $M_{1}{ }^{\prime}$ | $\sigma^{2}+\mathrm{r} \sigma^{2}{ }_{\text {gxe }}$ |
| $\mathrm{F}_{3} \times$ Env. | (e-1)(1-1) | $M_{1}$ | $\sigma^{2}+r \sigma_{\text {F }}{ }^{2 x x}$ |
| Residuals $\times$ Env. | $(\mathrm{e}-1)(\mathrm{g}-1)$ |  |  |
| Error | $e(r-1)(g-1)$ | $M_{1}$ | $\sigma^{2}$ |
| Total | zge-1 |  |  |

## ADJUSTED ENTRY MEANS IN LATTICE DESIGN

| Source | df | MS | EMS |
| :---: | :---: | :---: | :---: |
| Environments (Env.) | e-1 |  |  |
| Entries | $g-1$ | $\mathrm{M}_{2}{ }^{\prime}$ | $\sigma^{2}+r \sigma^{2}{ }_{\text {pre }}+r e \sigma_{g}^{2}$ |
| $\mathrm{F}_{3}$ Lines | 1-1 | $M_{2}$ | $\sigma^{2}+r \sigma_{\text {F3xe }}{ }^{2}+\mathrm{re} \sigma_{\mathrm{F} 3}{ }^{2}$ |
| Residuals | g-1 |  |  |
| Entries $x$ Env. | $(e-1)(g-1)$ | $M_{1}{ }^{\text {, }}$ | $\sigma^{2}+r \sigma^{2}$ |
| F3 $\times$ Env. | $(e-1)(1-1)$ $(e-1)(\mathrm{g}-1)$ | $M_{1}$ | $\sigma^{2}+r \sigma_{F 3 x 0}^{2}$ |

Total

## APPENDIX 9. ANALYSIS OF VARIANCE OF THE REGRESSION MODEL FOR SINGLE MARKER ANALYSIS

| Source | d | $\mathrm{M} . \mathrm{S}$. |
| :--- | :---: | :---: |
| Regression | 2 | $\mathrm{MS}_{\mathrm{R}}$ |
| Additive (A-B) | 1 | $\mathrm{MS}_{\text {ndd. }}$ |
| Dominance $\left[\frac{1}{2}(\mathrm{~A}+\mathrm{B})-\mathrm{H}\right]$ | 1 | $\mathrm{MS}_{\text {dam. }}$ |
| Error |  | $\mathrm{MS}_{\mathrm{r}}$ |

APPENDIX 10. RESULTS FROM SINGLE MARKER ANALYSIS^


$17588.2(0.0001)$
11977.1 (0.0001)
17545.7 (0.0001) 11400.7 (0.0002)
26756.9 (0.0001)
25826.1 (0.0001)

Anthesis, GDD, Env. 3


| 5 | BNL5. 71 | 13313.49 | (0.0045) | 464.14 (0.6588) | 26095.90 | (0.0012) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 5 | UMC51 | 8128.02 | (0.0361) | 151.22 (0.8019) | 16157.88 | (0.0103) |
| 8 | NPI268 | 41238.01 | (0.0001) | 9879.83 (0.0268 | 79030.79 | (0.0001) |
| 8 | UMC89 | 43396.67 | (0.0001) | 21983.57 (0.0006) | 74893. | $84(0.0001)$ |
|  | (GDD), Env. 2 |  |  |  |  |  |
| 2 | UMC 53 | 10197.38 | (0.0062) | 13151.66 (0.0101) | 11042.23 | (0.0181) |
| 2 | UMC78 | 8017.31 | (0.0202) | 12899.06 (0.0121) | 6181.25 | (0.0808) |
| 3 | UMC16 | 12013.92 | (0.0027) | 23379.46 (0.0007) | 6.79 | (0.9530) |
| 3 | ISU1 | 12846.13 | (0.0018) | 24586.88 (0.0005) | 13.45 | (0.9337) |
| 8 | NPI268 | 20972.42 | (0.0001) | 18433.60 (0.0020) | 29457.50 | (0.0001) |
| 8 | UMC89 | 24712.93 | (0.0001) | 16598.89 (0.0026) | 39375.67 | (0.0001) |
| 10 | PIO20.0075 | 3259.76 | (0.2117) | 4998.75 (0.1230) | 2312.76 | (0.2931) |
| 10 | UMC64 | 316.86 | (0.8601) | 84.90 (0.8409) | 620.51 | (0.5876) |
|  | Silk Emergence (GDD), Env. 1 |  |  |  |  |  |
| 1 | UMC157 | 72713.46 | (0.0004) | 10286.88 (0.2801) | 142908. 15 | (0.0001) |
| 1 | NPI234 | 159969.88 | (0.0002) | 4790.42 (0.4652) | 159938.32 | (0.0001) |
| 2 | UMC53 | 39209.36 | (0.0166) | 30569.83 (0.0720) | 61413.69 | (0.0112) |
| 2 | UMC78 | 67408.10 | (0.0007) | 38362.99 ( 0.043) | 118890.18 | (0.0003) |
| 5 | BNL5.71 | 53194.37 | (0.0036) | 77.06 (0.9267) | 100018.97 | (0.0011) |
| 5 | UMC51 | 69040.51 | (0.0006) | 20549.35 (0.1274) | 131406.93 | (0.0002) |
| 8 | NPI268 | 84518.25 | (0.0001) | 45438.14 (0.0212) | 143708.63 | (0.0001) |
| 8 | UMC89 | 95044.34 | (0.0001) | 61618.41 (0.0075) | 152982.16 | (0.0001) |
|  | Silk Emergence (GDD), Env. 2 |  |  |  |  |  |
| 1 | BNL8. 29 | 62717.20 | (0.0003) | 92456.52 (0.0005) | 56507.16 | (0.0063) |
| 1 | BNL15. 18 | 87731.78 | (0.0001) | 66915.98 (0.0022) | 136511.63 | (0.0001) |
| 1 | NPI234 | 57884.39 | (0.0008) | 23.21 (0.9564) | 111938.89 | (0.0002) |
| 1 | UMC11 | 18893.64 | (0.0729) | 9.41 (0.9710) | 36681.27 | (0.0244) |
| 6 | UMC51 | 2133.98 | (0.3609) | 0.52 (0.9874) | 4167.66 | (0.1590) |
| 6 | UMC68 | 46979.16 | (0.0027) | 22841.11 (0.0853) | 82040.89 | (0.0013) |
| 8 | NPI268 | 53832.63 | (0.0011) | 16282.52 (0.1423) | 100980.03 | (0.0003) |
| 8 | UMC89 | 33538.19 | (0.0136) | 10561.09 (0.2397) | 62719.48 | (0.0046) |
| 9 | PIO10.5 | 56922.61 | (0.0008) | 686.35 (0.7636) | 111173.65 | (0.0002) |
| 9 | CI | 38753.91 | (0.0075) | 4916.61 (0.4246) | 77455.05 | (0.0018) |
|  | Plant Height, Env. 1 |  |  |  |  |  |
| 1 | BNL15.18 | 1927.35 | (0.0001) | 1979.53 (0.0012) | 2695.35 | (0.0002) |
| 1 | UMC128 | 1416.12 | (0.0012) | 1000.53 (0.0272) | 2441.67 | (0.0007) |
| 3 | UMC60 | 1042.21 | (0.0068) | 98.76 (0.4854) | 1784.40 | (0.0035) |
| 3 | UMC165 | 1286.50 | (0.0019) | 16.38 (0.7737) | 2437.24 | (0.0006) |
| 6 | Purple | 1572.75 | (0.0005) | 9.83 (0.8240) | 2914.96 | (0.0002) |
| 6 | UMC85 | 597.72 | (0.0598) | 199.44 (0.3292) | 1183.50 | (0.0184) |
|  | Plant Height, Env. 2 |  |  |  |  |  |
| 1 | BNL15.18 | 3928.43 | (0.0001) | 5931.17 (0.0001) | 3206.06 | (0.0001) |
| 1 | UMC128 | 1489.93 | (0.0010) | 1772.73 (0.0039) | 1856.23 | (0.0032) |
| 10 | PIO20.0075 | 545.99 | (0.0827) | 593.60 (0.0990) | 644.08 | (0.0858) |
| 10 | UMC64 | 405.96 | (0.1584) | 25.41 (0.7330) | 809.72 | (0.0556) |
|  | Ear Height, Env. 1 |  |  |  |  |  |
| 1 | BNL8. 29 | 661.16 | (0.0005) | 33.82 (0.5243) | 1319.22 | (0.0001) |
| 1 | BNLI5. 18 | 681.53 | (0.0003) | 772.63 (0.0021) | 884.81 | (0.0010) |
|  | Ear Height, Env. 2 |  |  |  |  |  |
| 1 | BNL15.18 | 770.52 | (0.0001) | 753.19 (0.0014) | 1050.96 | (0.0002) |
| 1 | UMC128 | 455.99 | (0.0030) | 587.45 (0.0059) | 522.74 | (0.0093) |
| 5 | BNL8. 33 | 386.86 | (0.0078) | 362.06 (0.0319) | 580.90 | (0.0068) |
| 5 | BNL6. 25 | 446.81 | (0.0036) | 536.43 (0.0089) | 510.03 | (0.0107) |
| 7 | UMC80 | 500.82 | (0.0019) | 1001.07 (0.0004) | 91.30 | (0.2763) |
| 7 | BNL8. 39 | 48.41 | (0.5551) | 52.74 (0.4236) | 65.90 | (0.3712) |




| 5 | BNL5. 71 | 0.09 | (0.0052) | 0.11 | (0.0104) | 0.12 | (0.0078) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 10 | NPI232 | 0.10 | (0.0030) | 0.18 | (0.0011) | 0.04 | (0.1085) |
| 10 | NPI287 | 0.03 | (0.1293) | 0.07 | (0.0454) | 0.01 | (0.4675) |
| 1 | BNL8. 29 | 0.28 | $\begin{gathered} \text { Kernel } \\ (0.0007) \end{gathered}$ | $\begin{array}{r} \text { Env. } 2 \\ 0.50 \end{array}$ | (0.0003) | 0.14 | (0.0498) |
| 1 | BNL15.18 | 0.07 | (0.0176) | 0.38 | (0.0010) | 0.52 | (0.0001) |
| 3 | UMC175 | 0.19 | (0.0085) | 0.04 | (0.2995) | 0.36 | (0.0024) |
| 3 | UMC50 | 0.20 | (0.0054) | 0.08 | (0.1565) | 0.38 | (0.0019) |
| 9 | PIO10.5 | 0.20 | (0.0074) | 0.03 | (0.3480) | 0.39 | (0.0018) |
| 9 | CIC9S | 0.25 | (0.0018) | 0.01 | (0.5666) | 0.49 | (0.0004) |

A Summarized results from single marker analysis described in materiala and method. The analysis folloewd the format illustrated in Appendix 9.

APPENDIX 11. CLIMATOLOGICAL DATA FOR 1990 AND 1991 IN AGRONOMY FARM IN AMES
Temperature ( $\mathrm{F}^{\circ}$ )*


|  | GDD |  |  |
| :--- | ---: | ---: | ---: |
|  |  |  |  |
|  | 1990 | 1991 | Normal* |
| April | 220.0 |  |  |
| May | 529.5 | 204.0 | 576.3 |
| June | 1150.5 | 676.0 | 1158.0 |
| July | 1831.5 | 1389.0 | 1864.2 |
| August | 2508.5 | 2067.0 | 2516.7 |
| September | 3053.5 | 2729.0 | 2941.6 |
| * average over last 30 years |  | 3218.0 |  |


[^0]:    A coefficient of variation
    ${ }^{B}$ Probability of $W$ smaller than standard table value

[^1]:    A coefficient of variation
    ${ }^{B}$ Probability of $W$ smaller than standard table value

[^2]:    * significant at 0.05 level
    *significant at 0.01 level

[^3]:    A coefficient of variation
    B Probability of $W$ smaller than standard table value

[^4]:    * significant at 0.05 level ** significant at 0.01 level

[^5]:    ${ }^{\wedge}$ Numbers in the brackets were the probability of significant test for correlation coefficients

[^6]:    Unadi.
    ASL
    

[^7]:    
    
    

[^8]:    APPENDIX 7. DISTRIBUTION OF TRAITS

