

## Quantitative Trait Loci for Leaf Angle, Leaf Width, Leaf Length, and Plant Height in a maize (*Zea mays* L) B73 × Mo17 population

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### Abstract

Morphological traits affecting the canopy architecture of maize (*Zea mays* L) in high density cultivation can affect productivity. To identify quantitative trait loci affecting some of these traits, including leaf angle, leaf width, leaf length, and plant height, I analyzed these traits in the intermated B73 × Mo17 recombinant inbred line population, IBM-94, a maize population that other researchers developed for genetic studies by the scientific community. I collected data for the traits in replicated trials of IBM-94 grown in 2007, 2008, and 2009 at Sioux Falls, SD, USA. Means  $\pm$  standard errors were for leaf angle =  $26 \pm 2.6^\circ$ , leaf width =  $91.0 \pm 2.95$  mm, leaf length  $764.6 \pm 20.76$  mm, plant height =  $2231.3 \pm 101.24$  mm. There was significant ( $\alpha = 0.01$ ) genotypic variation for each trait. Heritability for height = 74.5%, leaf width = 86.2%, leaf length = 85.3%, and leaf angle = 90.5%. Phenotype data were combined with genetic maps and molecular marker haplotypes from the Maize Genetics and Genomics Database to analyze for quantitative trait loci by composite interval mapping using PLABMQTL computer software. One to three quantitative trait loci with logarithm of odds equivalent to  $\alpha = 0.05$  were identified for each trait. Three quantitative trait loci were identified for leaf angle on chromosomes 1, 5, and 9, two for leaf width on chromosomes 2 and 8, one for leaf length on chromosome 2, and one for plant height on chromosome 4. Regression models explained 27.1% of leaf angle, 25.0% of leaf width, 9.1% of leaf length, and 8.4% of plant height variation.

**Keywords:** leaf angle, leaf width, leaf length, plant height, QTL

### Introduction

Modern maize hybrid varieties have steadily become more productive through the past several decades. The increased productivity is partly due to higher population densities and genetic adaptations that permit vigorous growth at high densities. Because efficient light interception is essential to plant growth, plant forms that enable efficient light interception in high population densities will increase yield production under modern field conditions. A near vertical leaf angle was one of several traits included in an ideotype of maize that Mock and Pearce (1975) proposed as a plant breeder's ideal when developing maize varieties for high population density. Comparing normal and near-isogenic *liguless-2* hybrids, those with the *liguless-2* gene had a more upright leaf than the normal hybrid and yielded more grain with 90,000 or 75,000, but not 60,000 plant per ha population density (Lambert and Johnson, 1978). Leaf angle and plant height were two factors that significantly changed with generation of Pioneer brand hybrids and parental lines from the 1930s to 2000 (Lauer et al, 2012). Plant size could influence grain yield by apportioning no more sugar to stalk production than is needed for robust growth and light interception. Maize plants allocating no more sugar than needed to stable structures of the stalk, which have little potential for mobilizing sugars for relocation to grain, could translocate more sugar to developing grain (Mock and Pearce, 1975). Leaf length and width of Pioneer

brand hybrids did not significantly vary over generations (Lauer et al, 2012). Nevertheless, one may reasonably speculate that in some exotic populations or populations derived from crosses between highly divergent parents, variation for leaf length and width might affect light interception and productivity. Traits with continuous or non-discrete classes are typically controlled by multiple genes called quantitative trait loci (QTL). Because the inheritance of such traits may be complex, knowing where QTL are located can be useful for genetic improvement of quantitative traits. To identify QTL affecting leaf traits and plant height in maize I performed a genetic analysis in IBM-94, a recombinant inbred line (RIL) population. IBM-94 is a subset of IBM-302, which is 302 RIL derived from the intermated B73 × Mo17 RIL population developed by other researchers for genetic studies (Lee et al, 2002; Coe et al, 2002; Cone et al, 2002).

### Materials and Methods

The population for study was IBM-94. Because resources were limited, IBM-94 was used instead of IBM-302. Seed for the 93 RIL constituting IBM-94 was obtained from the Maize Genetics Cooperative Stock Center (<http://maizecoop.cropsci.uiuc.edu>). In year 2006 the original seed was grown and plants were self-pollinated to produce enough RIL seed to plant replicated experimental plots at Sioux Falls, SD, USA. In years 2007, 2008, and 2009 the 93 RIL were grown in randomized complete blocks with three replicates

**Table 1** - Some leaf morphology traits and plant height of IBM-94 RIL grown at Sioux Falls, SD in 2007, 2008, and 2009.

|                               | Leaf Angle   |         | Leaf Width    |        | Leaf Length     |         | Plant Height       |         |  |  |  |  |  |  |
|-------------------------------|--------------|---------|---------------|--------|-----------------|---------|--------------------|---------|--|--|--|--|--|--|
|                               | Degrees      |         | mm            |        |                 |         |                    |         |  |  |  |  |  |  |
| RIL Mean <sup>†</sup>         | 26 ± 2.6     |         | 91.0 ± 2.95   |        | 764.6 ± 20.76   |         | 2,231.3 ± 101.24   |         |  |  |  |  |  |  |
| Range among RILs <sup>‡</sup> | 9 to 53      |         | 71.2 to 114.5 |        | 641.7 to 907.0  |         | 1,323.2 to 2,796.8 |         |  |  |  |  |  |  |
| ANOVA                         |              |         |               |        |                 |         |                    |         |  |  |  |  |  |  |
| Source of Variation           | MS, F        |         |               |        |                 |         |                    |         |  |  |  |  |  |  |
| Genotypes                     | 623.8        | 10.49** | 576.6         | 7.24** | 26,374          | 6.80**  | 362,149            | 3.93**  |  |  |  |  |  |  |
| Years                         | 1026         | 9.54*   | 990.6         | 3.31   | 582,080         | 35.41** | 18,780,568         | 71.16** |  |  |  |  |  |  |
| Genotype × Year               | 59.5         | 1.68**  | 79.6          | 1.52** | 3,878           | 1.86**  | 92,242             | 4.27**  |  |  |  |  |  |  |
| Replications in Years         | 107.5        | 3.04**  | 299.2         | 5.72** | 16,436          | 7.87**  | 263,924            | 12.21** |  |  |  |  |  |  |
| Error                         | 35.3         |         | 52.3          |        | 2,089           |         | 21,617             |         |  |  |  |  |  |  |
| Variance Components and $h^2$ |              |         |               |        |                 |         |                    |         |  |  |  |  |  |  |
| $\sigma^2_g$                  | 62.7 ± 10.13 |         | 55.2 ± 9.40   |        | 2,500 ± 429.8   |         | 29,990 ± 5,965.4   |         |  |  |  |  |  |  |
| $\sigma^2_e$                  | 3.3 ± 2.61   |         | 2.5 ± 2.57    |        | 2,027 ± 1,475.5 |         | 66,368 ± 47,600.4  |         |  |  |  |  |  |  |
| $\sigma^2_{ge}$               | 8.1 ± 2.19   |         | 9.1 ± 2.96    |        | 596 ± 141.2     |         | 23,542 ± 3,226.4   |         |  |  |  |  |  |  |
| $\sigma^2_{rep(e)}$           | 0.8 ± 0.58   |         | 2.7 ± 1.61    |        | 154 ± 88.4      |         | 2,605 ± 1,419.0    |         |  |  |  |  |  |  |
| $\sigma^2_{error}$            | 35.3 ± 2.14  |         | 52.3 ± 3.16   |        | 2,089 ± 126.2   |         | 1,617 ± 1,304.8    |         |  |  |  |  |  |  |
| $h^2$ , %                     | 90.5         |         | 86.2          |        | 85.3            |         | 74.5               |         |  |  |  |  |  |  |
| $h^2$ , 90% confidence int    | 86.24, 93.26 |         | 80.08, 90.23  |        | 78.78, 89.59    |         | 63.25, 81.97       |         |  |  |  |  |  |  |

\*,\*\* Significant at  $\alpha = 0.05, 0.01$ , respectively; <sup>†</sup> ± Standard error

each year. Each plot included four plants spaced 279 mm apart in rows 762 mm apart with no spaces between plots within rows. At anthesis the total plant height to the tassel tip and the leaf at the uppermost ear shoot were measured on the two center plants in each plot. Leaf measurements included the leaf angle from vertical, maximum leaf width, and distance from the ligule to the tip of the straightened leaf.

Statistical analysis, including analysis of variance (ANOVA) and heritability ( $h^2$ ) was performed with PLABSTAT (University of Hohenheim, Germany, <https://plant-breeding.uni-hohenheim.de/~ipspwww/soft.html>). Years and replications were random effects and genotype was a fixed effect in the ANOVA. Heritability was calculated by PLABSTAT on an entry mean basis using mean squares from the ANOVA as described by Knapp et al (1985). Because the genotypes were RIL,  $h^2$  is narrow-sense. Genetic maps of markers and molecular marker genotypes of each RIL were obtained from the Maize Genetics and Genomics Database (MaizeGDB, <http://www.maizegdb.org>).

Phenotype data for the RILs were combined with marker genotypic information and map distances from the MaizeGDB IBM2 map to analyze for QTL by composite interval mapping using PLABMQTL (University of Hohenheim, Germany, <https://plant-breeding.uni-hohenheim.de/~ipspwww/soft.html>). The QTL included in the scans and multiple regression models were limited to those detected with logarithm of odds (LOD) thresholds equivalent to an  $\alpha = 0.05$  genome-wide error rate, as described by Cassady et al (2001). The LOD thresholds were determined by testing 1000 permutations of the data. The LOD thresholds equivalent to  $\alpha = 0.05$  were for leaf angle = 3.43, leaf width = 3.27, leaf length = 3.25, and plant height = 3.56. Cofactors for composite interval mapping (CIM) were selected by PLABMQTL in a preliminary analysis.

The most informative four to six cofactors in the preliminary selection step were empirically selected to maximize model informativeness as indicated by the coefficient of determination adjusted for the number of terms in the model ( $R^2_{adj}$ ), and these cofactors were used in the final analyses. Cross validation with the data set randomly divided among lines in 200 five-fold detection and validation runs with one-fifth of the data set tested against the remaining four-fifths of the data set was employed to indicate whether bias in the data influenced identification of QTL (Utz et al, 2000).

## Results and Discussion

There was significant variation among RIL (Table 1) for all traits. Heritabilities ranged from 74.5% for plant height to 85.3% for leaf length, 86.2% for leaf width, and 90.5% for leaf angle. Some of the RIL were intolerant of water stress as indicated by leaf rolling and delayed silking. The water stress sensitivity probably was confounded with the potential for elongation of growing cells, ultimately affecting plant height and leaf size, and might have increased random variation. Plant height and leaf length were more strongly influenced by environment than were leaf width and leaf angle, since years were a highly significant ( $\alpha = 0.01$ ) source of variation for plant height and leaf length, significant ( $\alpha = 0.05$ ) for leaf angle, and not significant for leaf width. The F test of the genotype × environment interaction ( $F_{GE}$ ) was highly significant for all traits with the leaf angle  $F_{GE} = 1.68$ , leaf width  $F_{GE} = 1.52$ , and leaf length  $F_{GE} = 1.86$ . And plant height  $F_{GE} = 4.27$  was the largest, indicating the probability of genotype × environment interaction was somewhat greater for plant height than the other traits.

One to three QTL were identified for each of the measured traits in IBM-94 (Table 2). The leaf angle

**Table 2** - Regression models with QTL for leaf angle, leaf width, leaf length, and plant height in IBM94 RILs. The QTL were detected and included in regression models if LOD values in scans were greater than the LOD threshold corresponding to  $\alpha = 0.05$ , by permutation test. Effects that are positive in a sign are favored by the Mo17 allele.

| Bin                 | Marker interval   | Chromosome and position | CV <sup>‡</sup> | Support Interval <sup>§</sup> | LOD  | Partial R <sup>2</sup> | Effect <sup>¶</sup> | R <sup>2</sup> <sub>adj</sub> <sup>#</sup> | ^ p <sup>††</sup> |
|---------------------|-------------------|-------------------------|-----------------|-------------------------------|------|------------------------|---------------------|--|-------------------|
| <b>Leaf Angle</b>   |                   |                         |                 |                               |      |                        |                     |  |                   |
| 1.05–1.05           | umc1603–uaz273    | 1/480                   | 59.8            | 465-495                       | 4.27 | 16.4%                  | -3.802              | 27.1%                                      | 32.5%             |
| 5.04–5.05           | csu308–umc1482    | 5/375                   | 44.6            | 345-390                       | 4.8  | 13.9                   | 3.084               |  |                   |
| 9.01–9.01           | umc1867–lim343    | 9/30                    | 62.9            | 15-45                         | 4.76 | 10.4                   | -2.76               |  |                   |
| <b>Leaf Width</b>   |                   |                         |                 |                               |      |                        |                     |  |                   |
| 2.04–2.04           | umc2088–umc2250   | 2/320                   | 86.5            | 300-340                       | 5.2  | 21.6                   | 4.076               | 25   | 31.2              |
| 8.03–8.03           | umc1735–php20714  | 8/280                   | 85.9            | 260-300                       | 5.28 | 15.2                   | -3.08               |  |                   |
| <b>Leaf Length</b>  |                   |                         |                 |                               |      |                        |                     |  |                   |
| 2.09–2.09           | bngl1893–AY110389 | 2/660                   | 54.4            | 640-680                       | 4.08 | 11.1                   | -21.531             | 9.1  | 11.9              |
| <b>Plant Height</b> |                   |                         |                 |                               |      |                        |                     |  |                   |
| 4.06–4.06           | umc2027–AY110310  | 4/350                   | 40.3            | 330-360                       | 4.01 | 10.4                   | -26.64              | 8.4  | 32.1              |

<sup>\*</sup>, <sup>\*\*</sup>  $\alpha = 0.05$  or  $0.01$ , respectively, for the probability that this QTL affected the trait independently of other QTL; <sup>†</sup>Bins where the flanking markers are located and positions are the coordinate values on the MaizeGDB IBM2 map (<http://www.maizegdb.org>); <sup>‡</sup>Frequency of detection within a 1-LOD support interval in 1000 cross validation runs with families randomly divided for detection and validation; <sup>§</sup>Interval with LOD scores within 1 LOD of the QTL peak; <sup>¶</sup>Effects were determined in a simultaneous multiple regression that included factors with LOD  $\geq$  the  $\alpha = 0.05$  threshold; <sup>#</sup>R<sup>2</sup> adjusted for the number of terms in the multiple regression models; <sup>††</sup>The proportion of genotypic variance explained by all QTL in the models.

regression model included QTL on chromosomes 1, 5, and 9, and R<sup>2</sup><sub>adj</sub> = 27.1%. The flanking markers for the leaf angle QTL are in bins 1.05, 5.04 and 5.05, and 9.01 of the MaizeGDB IBM2 map (<http://www.maizegdb.org>). These QTL are near several leaf angle QTL [Mickelson et al \(2002\)](#) identified in a different B73 x Mo17 population. Two of the QTL Mickelson et al found were in bins 5.03 and 5.06, thus near the QTL found in IBM-94 on chromosome 5 in the study reported here. And some QTL identified by [Ku et al \(2012\)](#) in a meta-analysis combining five QTL studies with six populations, included leaf angle QTL flanked by markers in bins 1.03, 1.09, and 5.01 and 5.06.

The regression model of leaf width in IBM-94 included QTL on chromosomes 2 and 8, and R<sup>2</sup><sub>adj</sub> = 25.0%. Based on bin locations of the flanking markers, 2.04 and 8.03, these are near two QTL [Reymond et al \(2004\)](#) identified for leaf width in a French flint x North American dent RIL population that had flanking markers in bins 2.03 and 2.04. Reymond also found more distant QTL with flanking markers in bins 2.06 and 8.06.

This study of IBM-94 identified one QTL for leaf length. Flanking markers are in bin 2.09, and R<sup>2</sup><sub>adj</sub> = 9.1%. [Reymond et al \(2004\)](#) identified several QTL for leaf length, including three on chromosome 2 with flanking markers in bins 2.04 and 2.06.

One QTL was identified for plant height in this study, with flanking markers in bin 4.06, and R<sup>2</sup><sub>adj</sub> = 8.4%. This is near a QTL for plant height with flanking markers in bin 4.05 [Veldboom and Lee \(1996\)](#) identified in a Mo17 x H99 population.

The larger IBM-302 RIL population and larger plots would have improved precision and might have enabled detection of more QTL. Cross validation for the plant height QTL (40.3%) was low indicating the

indicated QTL might not have had detectable influence in many RIL of IBM-94 and hence many of the test sets, each comprising one-fifth of the population, in validation. Because the IBM-94 population is a subset of 93 RIL from the larger IBM-302 population with 302 RIL the detection of QTL in test sets might have occurred with greater frequency for the IBM-302 population. Nevertheless, the IBM-94 population did enable detection of QTL, and some are near or at essentially the same locations as QTL for like traits identified by other researchers.

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