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Genetic Variation at *bx1* Controls DIMBOA Content in Maize

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1 The main hydroxamic acid in maize (*Zea mays* L.) is 2-4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA).
2 DIMBOA confers resistance to leaf-feeding by several corn borers. Most genes involved in the DIMBOA metabolic
3 pathway are located on the short arm of chromosome 4, and QTLs involved in maize resistance to leaf-feeding by
4 corn borers have been localized to that region. However, the low resolution of QTL linkage mapping does not allow
5 convincing proof that genetic variation at *bx* loci was responsible for the variability for resistance. This study
6 addressed the following objectives: to determine the QTLs involved in DIMBOA synthesis across genetically
7 divergent maize inbreds using eight RIL families from the NAM population, to check the stability of QTLs for
8 DIMBOA content across years by evaluating two of those RIL families in two years, and to test the involvement of
9 *bx1* by performing association mapping with a panel of 281 diverse inbred lines. QTLs were stable across different
10 environments. A genetic model including eight markers explained approximately 34% of phenotypic variability
11 across eight RIL families and the position of the largest QTL co-localizes with the majority of structural genes of the
12 DIMBOA pathway. Candidate association analysis determined that sequence polymorphisms at *bx1* greatly affects
13 variation of DIMBOA content in a diverse panel of maize inbreds, but the specific causal polymorphism or
14 polymorphisms responsible for the QTL detected in the region 4.01 were not identified. This result may be because
15 the causal polymorphism(s) were not sequenced, identity is masked by linkage disequilibrium, adjustments for
16 population structure reduce significance of causal polymorphisms or multiple causal polymorphisms affecting *bx1*
17 segregate among inbred lines.

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19 *Key words:* DIMBOA, QTL mapping, Association mapping, Nested association mapping, Candidate gene

1 Introduction

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Cyclic hydroxamic acids and their derivatives (benzoxazinoids) are major secondary metabolites among poaceous plants. These compounds have biological activity against plants, insects, fungi, and microorganisms (Niemeyer 1988; Pérez and Ormeño-Nuñez 1991) and could also be involved in detoxification of toxic inorganic molecules (Poschenrieder et al. 2005). The main hydroxamic acid in maize (*Zea mays* L.) is the 2- β -D-glucopyranosyloxy-4-hydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA-Glc) (Tipton et al. 1967; Cambier et al. 2000). The benzoxazinoid glucosides are stored in vacuoles as inactive phytoanticipines, while the glucosidases specific for their activation are present in the plastids (Babcock and Esen 1994; Czjzek et al 2000). Upon exogenous or endogenous damage to tissues, the glucoside comes in contact with the glucosidase and the toxic aglucone, DIMBOA (2-4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one), is released (Oikawa et al. 1999; von Rad et al. 2001; Park et al. 2004).

DIMBOA confers resistance to leaf-feeding by several corn borer species (Klun et al. 1967; Reid et al. 1991; Gutiérrez and Castañera 1986; Tseng 1997). DIMBOA decreases in-vivo endoproteinase activity in the larval midgut of the European corn borer (*Ostrinia nubilalis*) limiting the availability of amino acids and reducing larval growth (Houseman et al. 1989; 1992). DIMBOA has also been reported to affect detoxication and hydrolysis enzymes for Asian corn borer (*Ostrinia furnacalis*) and Mediterranean stem borer (*Sesamia nonagrioides*) (Yan et al. 1995; Ortego et al. 1998) larvae. However, DIMBOA had a differential impact on two closely related leaf-feeding generalists, *Spodoptera frugiperda* and *S. exigua* (Rostas 2007). *Spodoptera frugiperda*, which is native in the New World, copes well with DIMBOA in the diet, while DIMBOA is detrimental to *S. exigua*, an Asian species. Hedin et al. (1993) demonstrated that benzoxazinoids are also toxic factors for *Diatraea grandiosella*. The protection against insect attack that DIMBOA confers to the plant is restricted to early stages of plant development because DIMBOA concentration decreases with plant age (Morse et al. 1991; Barry et al. 1994; Cambier et al. 2000). Benzoxazinoids are considered as constitutive compounds, but their biosynthesis seems to be increased after insect damage or treatments with methyl jasmonate, a signal molecule of herbivore damage (Gutiérrez et al. 1988; Morse et al. 1991; Huang et al. 2006; Wang et al 2007). Richardson and Bacon (1993) suggested that growth limiting conditions, such as water deficit, also increases the accumulation of benzoxazinoids. Therefore, plant age, insect damage and certain environmental factors can alter DIMBOA levels.

In maize the genes involved in DIMBOA's metabolic pathway have been cloned and mapped (Jonczyk et al. 2008). Seven genes (*bx1*, *bx2*, *bx3*, *bx4*, *bx5*, *bx6*, and *bx8*) map to a genomic region of approximately 6 cM on the short arm of chromosome 4, *bx7* is also in the short arm of chromosome 4 and *bx9* is localized on chromosome 1. BX1 catalyzes the formation of free indole from indole-3-glycerol phosphate, then the stepwise action of four

1 maize cytochrome P-450-dependent monooxygenases (BX2, BX3, BX4, and BX5) convert free indole to DIBOA
2 (2-4-dihydroxy-1,4-benzoxazin-3-one) (Frey et al. 1997; Glawischnig et al. 1999). Sequence analysis indicates that
3 BX1 is a modified form of the tryptophan synthase alpha subunit (TSA). *bx1* is also evolutionary related to the gene
4 *igl* which codifies for indole-3-glycerol phosphate lyase, responsible for the formation of volatile indole (Frey et al.
5 2000; Gierl and Frey 2001). *bx1* is expressed constitutively in young seedlings, while *igl* is induced in more advanced
6 stages of plant development and contributes to the blend of odors that attract beneficial parasitoids. Therefore,
7 duplications of the *TSA* gene involved in the primary metabolism have played an important role in augmenting the
8 plant chemical-defense capacity against insect attack.

9 The next step in the metabolic pathway is the conversion of DIBOA to DIBOA-glc by the action of
10 specific glucosyltransferases. Two glucosyltransferases are responsible for the glucosylation of DIBOA, BX8 and
11 BX9 (von Rad et al. 2001). The conversion of DIBOA-glc to DIMBOA-glc requires hydroxylation and methylation.
12 BX6 is responsible for the hydroxylation step that converts DIBOA-glc to TRIBOA-glc (2,4,7-trihydroxy-2H-1,4-
13 benzoxazin-3(4H)-one) and this conversion likely takes place in the cytosol (Frey et al. 2003; Jonczyk et al. 2008).
14 Methylation is catalyzed by BX7, rendering DIMBOA-glc.

15 Quantitative trait locus (QTL) analysis has been used to study maize resistance to leaf-feeding by European
16 corn borer and other insect pests (Bohn et al. 2001; Jampatong et al. 2002; Brooks et al. 2005; 2007; Cardinal et al.
17 2006). There is only one report on the involvement of a QTL close to the *bx* gene cluster in resistance to leaf-
18 feeding by a native American lepidopteran species (Bohn et al. 2001). These results could be the consequence of
19 two important facts, DIMBOA may be non toxic to most native species (Rostas 2007) and/or materials used could
20 have low amounts of DIMBOA. On the contrary, all studies to localize QTLs involved in resistance to leaf-feeding
21 by European corn borer have found QTLs close to the region of chromosome 4 where most *bx* genes reside
22 (Jampatong et al. 2002; Cardinal et al. 2006). However, the low resolution of the QTL linkage mapping approach
23 does not allow convincing proof that genetic variation at *bx* loci was responsible for the resistance found in those
24 crosses. By contrast, association mapping is a fine mapping approach which enables researchers to look for
25 functional variation in a broader germplasm background (Zhu et al. 2008). Association mapping could be focused
26 on one or few candidate genes or on the scanning of the entire genome. Although the use of whole genome scan
27 association mapping to identify loci with major effect has been successful for some particular traits such as oleic acid
28 content in maize kernels (Beló et al. 2008), the candidate gene approach has been favoured in species with a fast
29 decay of linkage disequilibrium, such as maize. Association mapping studies with candidate genes have been
30 successful to locate polymorphisms in maize genes *bt2*, *sb1*, and *sb2* associated to kernel composition traits, in *ae1*
31 and *sb1* to amylose levels, in *vgt1* and *dwarf8* to flowering-time variation and in *c2* and *nhp1* to maysin content in the

1 silks (Wilson et al. 2004; Szalma et al. 2005; Camus-Kulandaivelu et al. 2006; Ducrocq et al. 2008). Recently, nested
2 association mapping (NAM) has been proposed as a powerful genome-wide association analysis tool to dissecting
3 the genetic basis of quantitative traits in species with low linkage disequilibrium (Yu et al. 2008; McMullen et al.
4 2009a; Buckler et al. 2009). NAM has an increased cost-effective power compared to conventional genome-wide
5 association mapping approaches because the highly dense map obtained for the founders of the RIL's populations
6 can be projected to the RILs by using common-parent-specific markers for genotyping founders and progenies.

7 Both linkage mapping and association approaches could be complementary when used to study maize
8 functional variation for DIMBOA content. Firstly, NAM is used to localize QTLs for DIMBOA content across
9 different RIL families. Then, if QTLs for DIMBOA content variation are identified in the proximity of *bx* genes,
10 the candidate gene approach will be used with *bx1* which is the committal step into the DIMBOA pathway and,
11 consequently, it is the prime candidate for affecting total DIMBOA accumulation. Association mapping will also be
12 performed with sequences from genes *bx2*, *bx3*, *bx4*, *bx5*, and *bx8* to check whether significant associations between
13 *bx1* polymorphisms and DIMBOA content could be consequence of linkage disequilibrium between *bx1* and the
14 other *bx* genes located in the same contig. *bx6* has been mapped near to *bx4* (Jonczyk et al. 2008), but
15 AY104457/PCO086194, which matches by BLAST to AF540907 (cDNA sequence for *bx6*) maps in chromosome 2
16 rather than in 4.01. Therefore, no amplicons could be obtained for *bx6* because there is not a reliable genomic
17 sequence for that gene. This study addressed the following objectives: to determine the QTLs involved in DIMBOA
18 synthesis across genetically divergent maize inbreds using eight RIL populations from the NAM, to check the
19 stability of QTLs for DIMBOA content across years by evaluating two of those RIL populations in two years, and
20 to study the involvement of *bx1* performing association mapping with a panel of 281 diverse inbreds.

21 22 **Material and Methods**

23 24 QTLs for DIMBOA across NAM families

25 *Plant material*

26
27 RIL families derived from B73 × Il14H and B73 × CML322 were evaluated in 2007 and 2008 for determining QTL
28 stability. Eight RIL families derived from crosses between the inbred line B73 and inbreds CML52, CML322,
29 IL14H, M37W, MS71, NC350, Oh43, and Tx303 were used to determine the QTLs involved in DIMBOA synthesis
30 across genetically divergent maize inbreds.

1 In summer of 2007 two RIL families of the NAM population, derived from crosses B73 × I14H and B73
2 × CML322, were evaluated for DIMBOA, DIMBOA-glc, and the sum of the two which we designate as DIMBOA-
3 (T) for total DIMBOA. Fifteen kernels per row were planted in 3.6 m rows, 0.9 m aisles, and 0.9 m between rows
4 on May 18 at the Agronomy Research Center near Columbia, MO. An augmented design was used for each RIL
5 family in which both parental lines were used as common testers across blocks of 20 RILs. Therefore, the inbred
6 B73 was also a common tester across RIL families. On June 25, 38 days after planting, two representative plants per
7 row were selected and a 5 cm section of whorl tissue centered at the highest ligule was collected. After discarding
8 the outside leaf the whorl sections from the two plants per row were bulked, lyophilized, ground and maintained at
9 – 20 °C until chemical analyses.

10 In 2008, eight RIL families derived from crosses between the inbred line B73 and inbreds CML52,
11 CML322, IL14H, M37W, MS71, NC350, Oh43, and Tx303 were evaluated for DIMBOA, DIMBOA-glc, and
12 DIMBOA-T. These eight RIL families are all also part of the NAM population (Yu et al. 2008; McMullen et al.
13 2009a) and include the two families evaluated in 2007. These plants were grown at Hinkson Bottoms within
14 Columbia MO. The kernels were planted on June 10 and whorl tissue collected on July 11, 32 days after planting.
15 Planting conditions and tissue handling were as in 2007.

16 In 2008, the day before tissue collection, the number of emerged leaves per plant was recorded on the two
17 plants per plot which were going to be collected. The fifth leaves had previously been marked 22 days after planting
18 to assure a proper leaf count.

19 20 *Chemical analyses*

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22 Chemical determinations of the amount of DIMBOA and DIMBOA-Glc molecules were performed using high
23 performance liquid chromatography (HPLC) using a modification of the procedure of Nakagawa et al. (1995).
24 Purified DIMBOA was a gift from Dr. Monika Frey, Technische Universität München, Freising, Germany.
25 DIMBOA-Glc was isolated from corn leaves and identified by its enzyme hydrolysis products. All other reagents
26 were ACS grade or better and purchased from Thermo-Fisher (St. Louis, MO). Since a DIMBOA-Glc standard was
27 not commercially available, DIMBOA-Glc concentration in samples was calculated based on DIMBOA standards
28 and adjusted for differences in molecular weights.

29 One hundred mg of freeze-dried ground whorl tissue was weighed into screw capped 15 mL polypropylene
30 Falcon tubes and 5 mL of HPLC grade methanol and 50 uL of acetic acid were added to each tube. The tubes were
31 vortexed and placed in a sonicator waterbath for 60 minutes at 60° C. The supernatant (0.5 mL) was combined with

1 0.5 mL distilled water in a microcentrifuge tube, vortexed, and centrifuged for 5 min at 13,000 rpm. The
2 supernatants were transferred into auto-sample vials for analysis by HPLC. The HPLC system consisted of a Hitachi
3 Model L-7100 pump with a Hitachi Model L-7400 UV detector (280 nm), Hitachi Model L-7200 autosampler with
4 Hitachi D-7000 data acquisition interface and ConcertChrom software on a microcomputer. The column was a 100
5 x 4.6 mm reversed-phase Luna 3 μ m C18 BDS analytical column (Phenomenex) fitted with a C₁₈ ODS
6 SecurityGuard 4.0 x 3.0 mm guard column (Phenomenex) with a mobile phase consisting of methanol:1% acetic
7 acid in water (20:80) run at a flow rate of 1 mL min.

8

9 *Statistical analyses*

10 To check the stability and precision of QTLs detected for DIMBOA content, linkage mapping was done
11 independently for each of the RIL families derived from B73 \times CML322 and B73 \times IL14H in each year (2007 and
12 2008) and across years. Original linkage maps for each population had more than 700 SNP markers
13 (www.panzea.org); but QTL analyses were performed using linkage maps with an average distance between loci of
14 about 10 cM, yielding a final set of 183 and 178 markers for the 185 and 194 RILs obtained from crosses B73 \times
15 CML322 and B73 \times IL14H, respectively. QTL Cartographer (Basten et al. 2005) was used for cofactor selection
16 using a *p*-value for the partial F statistic of 0.01. A LOD threshold of 3.0 was chosen for declaring the putative
17 QTL significant using composite interval mapping. The LOD of 3.00 is slightly above the score value obtained by
18 the permutation test method (Churchill and Doerge, 1994) yielding an experiment wise error rate of 25%.
19 PLABQTL (Utz and Melchinger 2003) was used to obtain a final simultaneous fit using as covariates those loci
20 detected by QTL Cartographer. In addition, all putative QTLs were examined for QTL \times environment interaction.
21 The proportion of phenotypic variance explained by all QTLs was determined by the adjusted coefficient of
22 determination of regression (R^2_{adj}) fitting a model including all detected QTLs. The proportion of genotypic
23 variance explained by all QTL for one trait (*p*) was calculated as $p = (\text{genetic variance explained by QTL effects} /$
24 $\text{genetic variance}) \times 100$.

25 Fivefold cross validation (CV/G) of QTLs was performed following the procedures described by Utz et al.
26 (2000). The whole data set was randomly split into $k=5$ data subsets. Four of these subsets were combined to form
27 the estimation set (ES) and the remaining subset formed the test set (TS) in which predictions derived from ES were
28 tested for their validity by correlating predicted and observed data. We used 1000 replicate CV/G runs. Estimates
29 of medians and percentiles and frequency of QTL detection in ES and TS were calculated over all replicated CV/G
30 runs. The PLABQTL (Utz and Melchinger 2003) software package was used for all calculations.

1 To assess the allelic effects of QTLs shared across families, QTL analyses for DIMBOA-(T) content were
2 made across the eight NAM RIL families (1524 RILs) from 2008 using a multiple regression approach with 1106
3 loci (Buckler et al. 2009). The NAM map used can be found at www.panzea.org. The PROC GLMSELECT
4 procedure of SAS (SAS, 2000) was used to choose co-factors using a probability level of 0.00001 for entering and
5 deleting factors (Buckler et al. 2009). Regression analysis was performed with those cofactors and the best fit model
6 for DIMBOA-(T) content was obtained by using the PROC GLM procedure of SAS.

7 8 Diversity analysis of the *bx* candidate genes 9

10 Two hundred and eighty one genetically diverse inbreds were evaluated in 2008 (see list at
11 http://www.panzea.org/lit/Basic281Inbreds_20081210.xls). The experimental design was a complete random
12 block design with two replications. The experimental plot was the same as for RIL evaluations. Thirty-five days
13 after planting, five cm of whorl tissue were collected from two plants per plot, bulked, lyophilized, ground and
14 maintained at – 20 °C until performing chemical analyses.

15 The DNA was extracted using standard protocols (Saghai-Marooof et al. 1984) with minor modifications.
16 The genomic DNA sequence X76713 (<http://www.ncbi.nlm.nih.gov>), corresponding to *bx1* was BLASTed against
17 the B73 sequence database (<http://www.maizesequence.org/index.html>) and an evidence-gene sequence,
18 AC200309.3:82911-85155bp (GRMZM2G085381), was identified. PCR primers to amplify four amplicons of 500-
19 800 bp (Supplemental Table 1) covering the evidence-gene from 36 bp downstream of the 5' end to 148 bp
20 upstream of 3' end were designed using the Primer3 program (<http://frodo.wi.mit.edu/>). Similarly, evidence-gene
21 sequences for *bx1*-adjacent *bx* genes (*bx2*, AC200309.3:87362-89517 (GRMZM2G085661); *bx3*, AC193441.3:7574-
22 10352 (GRMZM2G167549); *bx4*, AC213878.3:11418-114239 (GRMZM2G172491); *bx5*, AC213878.3:49953-52529
23 (GRMZM2G063756) and *bx8*, AC200309.3:36796-38707 (GRMZM2G085054)) were identified and PCR primer
24 pairs were designed to generate amplicons for each sequence. Touchdown PCR was performed using Promega or
25 Phire Taq in a DNA Engine Tetrad thermocycler (MJ Research). The PCR program consisted of one cycle of 1 min
26 at 94°C, 1 min at 65°C, and 1 min 30 sec at 72°C; the same cycle was repeated with 1°C decrement in annealing
27 temperature per cycle until annealing temperature is 55°C; then, 34 cycles of 1 min at 94°C, 1 min at 55°C, and 1
28 min 30 sec at 72°C are performed. Following PCR amplification, unincorporated primers and deoxynucleotide
29 triphosphates were removed by ethanol precipitation prior to sequencing. For each amplicon, the PCR products
30 were sequenced with forward and reverse primers using BigDye terminator version 3.1 terminator cycle sequencing
31 kit (Applied Biosystems) and analyzed on an ABI 3700 sequencer (Applied Biosystems).

1 Base calling, quality assessment, and trimming of trace files were conducted with PHRED (Ewing and
2 Green, 1998; Ewing et al., 1998), and sequence assembly was performed by PHRAP. The multiple sequences for
3 each gene were aligned with DNAAlignEditor (Sanchez-Villeda et al. 2008) and edited manually. All sequences are
4 available from GenBank with accessions GF098181-GF099430 and GF100745-GF101806 and association mapping
5 and linkage disequilibrium (LD) analyses were performed using TASSEL version 2.1 (Bradbury et al. 2007).
6 Polymorphisms with low allele frequency (< 5%) were removed. Association analyses were performed by using
7 generalized linear (GLM) and mixed linear (MLM) regression models accounting for population structure (GLM and
8 MLM) and relatedness among individuals (MLM). Population structure was controlled using both the (Q) and
9 kinship (K) matrixes as reported by Flint-García et al. (2005) and Yu et al. (2006), respectively.

11 Results

13 *Mapping of QTLs across years*

15 Linkage mapping of RIL families derived from B73 × CML322 and B73 × IL14H for DIMBOA, DIMBOA-Glc,
16 and DIMBOA-(T) content was performed across years (2007 and 2008). The parents of the (B73 × CML322) RIL
17 family differed for DIMBOA, DIMBOA-Glc, and DIMBOA-(T) content in 2007, but not in 2008 (Table 1).
18 Similarly, the parents of the (B73 × IL14H) RIL family differed for the three fractions in 2007, but only for
19 DIMBOA content in 2008. Heritabilities for DIMBOA, DIMBOA-Glc, and DIMBOA-(T) contents were high in
20 both RIL families (Table 1).

21 QTLs affecting DIMBOA, DIMBOA-Glc and DIMBOA-(T) contents across years were detected in both
22 RIL families (Tables 2 and 3). In general, QTLs detected for DIMBOA-(T) co-localized with QTLs detected for
23 either DIMBOA and/or DIMBOA-Glc. Five QTLs were found for DIMBOA-(T) in the RILs derived from B73 ×
24 CML322 (Table 2). The largest QTL was detected on the short arm of chromosome 4, explaining the 42.5 % of the
25 phenotypic variance for DIMBOA-(T). The favourable allele was supplied by the inbred B73. Two other major
26 QTLs on chromosomes 1 and 8, explained 18.4 and 13.7 % of the phenotypic variability, respectively. Favourable
27 alleles for these two QTL were also contributed by B73. Favourable alleles for minor QTLs situated in
28 chromosomes 3 and 7 were inherited from the parental inbred with low DIMBOA-(T) content, CML322. The final
29 model explained 55.3% of genetic variance. In cross validation analysis, the median QTL effect for the QTL on
30 chromosome 4 calculated from CV/G was similar to the value calculated from the full data set. However, for the
31 other QTLs, the ratio between CV/G and full data set varied from 0.80 to 2.40. QTLs located on chromosomes 1,

1 3, 4, 7, and 8 were detected in 97.2%, 79.0%, 100.0%, 82.1%, and 62.0% of all cross validations runs, and explained
2 almost 50% of the genetic variance.

3 Three QTLs were found for DIMBOA-(T) content in the RIL family obtained from B73 × II14H (Table
4 3). The most important QTL was again localized at the beginning of chromosome 4 and explained approximately
5 the 9.5% of phenotypic variability. The favourable allele for this QTL was derived from the inbred II14H. The two
6 other QTLs were located in chromosomes 1 and 6. B73 supplied the favourable allele for the QTL in chromosome
7 1 and II14H for the QTL in chromosome 6. In this particular RIL family, QTL × environment effects were very
8 important, and the QTL detected only explained 5.4% of genetic variability. Again, the median QTL effect for the
9 QTL in chromosome 4 calculated from CV/G runs approximated the value calculated from the full data set. The
10 ratio between CV/G and full data set for QTLs in chromosomes 1 and 6 varied from 1.20 to 1.40. QTLs in
11 chromosomes 4 and 6 were detected in 76.5 and 80.4% of CV/G runs, but the QTL on chromosome 1 was only
12 detected in 26.7% of CV/G runs. The analysis of two years data for two RIL families indicated that shared QTLs
13 are present on chromosomes 1 and 4. The results also suggest that an allelic series exists for the chromosome 4
14 QTL with II14H > B73 > CML322.

16 *Mapping of QTLs across RIL families*

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18 To further test for shared QTL and the presence of allelic series for DIMBOA QTL eight RIL families were
19 evaluated in 2008 (Table 4). Mean values for each genotype were not adjusted by the block effect because there
20 were not significant differences among blocks for DIMBOA-(T) content in any experiment (data not shown).
21 Differences for DIMBOA-(T) were significant between the parents of the RILs derived from B73 × CML52, B73 ×
22 MS71, and B73 × NC350. The mean of the DIMBOA-(T) amounts of RILs within a family, varied from
23 approximately the value of the high parent, intermediate between both parents or similar to the parent with the low
24 amount (Supplemental Table 2). The average of the RIL family derived from B73 × CML322 for DIMBOA-(T)
25 content was significantly lower than the mean of either parent.

26 From the GLMSELECT analysis across the eight families, QTLs with significant effects for DIMBOA-(T)
27 content were found on chromosomes 1, 2, 4, 5, 6, and 8 (Table 4). After accounting for the variability explained by
28 differences among families (family as a term in the model), the closest marker to the major QTL detected in
29 chromosome 4 explained more than 15% of phenotypic variation across the eight families. Each of the closest
30 markers to QTLs in chromosomes 2 and 5 explained more than 3% of phenotypic variability and each of the

1 remaining markers explained from 1.6 to 2.8% of the phenotypic variability across all families. The final model
2 explained approximately 34% of intra-population phenotypic variability across the eight families.

3 For DIMBOA-(T), significant effects of markers PZA03189 (chromosome 1, at bin 1.04), PHM1184
4 (chromosome 4, at bin 4.01), PZA01527 (chromosome 6, at bin 6.01), and PZA00473 (chromosome 6, at bin 6.05),
5 displayed both positive (favourable allele came from the non-B73 parent) or negative (favourable allele came from
6 B73) effects, depending on the specific RIL family (Table 4). Significant effects of the markers PZA00635
7 (chromosome 2, at bin 2.04) and PZA02746 (chromosome 8, at bin 8.06) were always negative, while significant
8 additive effects of markers PZA02002 (chromosome 4, at bin 4.04) and PZA00980 (chromosome 5, at bin 5.07)
9 were always positive. The effects of the marker PHM1184.26 were significant in seven out of the eight RIL families,
10 PZA03189.4 in six, PZA00473 and PZA02746 in five, PZA00635 in four, PZA00980 and PZA01527 in three, and
11 PZA02002 in two. The additive genetic effect of the marker PHM1184 was more than 1000 ppm in two families,
12 while genetic effects of markers PZA03189 (chromosome 1, at bin 1.04), PZA00635 (chromosome 2, at bin 2.04),
13 PZA00980 (chromosome 5, at bin 5.07) reached 600 ppm in specific families (Table 4).

14 As DIMBOA content declines with plant age, faster growing plants may have a lower DIMBOA content
15 by dilution of DIMBOA with greater plant mass. To determine if plant growth is a confounding factor for any of
16 the QTL reported above we determined the correlation of leaf number with DIMBOA levels and mapped QTL for
17 leaf number present at time of tissue collection. The number of leaves was significantly and negatively correlated
18 with DIMBOA-(T) content across populations ($r = -0.19$, $p < 0.0001$), and within the RIL families derived from the
19 crosses B73 \times CML52, B73 \times M37W, B73 \times MS71, and B73 \times Oh43, although correlation coefficients were low
20 (Supplemental Table 2).

21 As a significant relationship was found between DIMBOA-(T) content and number of leaves per plant,
22 QTL analyses for leaf number were performed. Three minor QTLs were detected explaining approximately the 9%
23 of variability for number of leaves. Those markers were PZA0300.2 located in chromosome 1 at bin 1.05,
24 PZA00485.2 located in chromosome 2 at bin 2.05, and PZB02044.1 in chromosome 3 at bin 3.05.

25 Diversity analysis of the *bx* candidate genes

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28 We identified 45 INDELs and 44 SNPs across the four amplicons in *bx1*, 6 INDELs and 11 SNPs in one amplicon
29 for *bx2*, 3 INDELs and 19 SNPs in one amplicon for *bx3*, 3 INDELs and 1 SNP in one amplicon for *bx4*, 10
30 INDELs and 35 SNPs across the three amplicons in *bx5*, and no polymorphisms of greater than 5% frequency for
31 *bx8*. Twenty-eight polymorphisms across *bx1* and one polymorphism in *bx2* were significantly associated ($p <$

1 0.001) with DIMBOA-(T) content in analysis using both Q + K matrix (Yu et al. 2006) to adjust for population
2 structure (Fig. 1) . The most strongly associated polymorphism was an SNP (A/G) at 620 bp in amplicon X76713.5
3 (X76713.5_620) of the gene *bx1*, Genetic variation at that polymorphism explained 4% of the phenotypic variation
4 not accounted for by population structure using MLM. Considering all polymorphisms in *bx1* significantly
5 associated to DIMBOA-(T), more than 50 different haplotypes were identified for *bx1* (supplemental table 3).
6 Therefore, in order to fit a multilocus model, we performed three-way ANOVA analyses including as sources of
7 variation: groups (Stiff Stalk, Non Stiff Stalk and Tropical – equivalent to Q), the X76713.5_620 polymorphism (as
8 being the most significantly associated to DIMBOA-(T) content) and then test all other significant polymorphism.
9 The model including the polymorphisms in exons 5 and 6 at sites X76713.5_620 and bx1_1.9_143 (3 bp INDEL at
10 143 bp in amplicon bx1_1.9), respectively, and variation among groups was the only model in which both
11 polymorphisms remained significant. This model explained 12% of variation not accounted for by population
12 structure and 27% of total variability among inbreds. Average DIMBOA-(T) content for each haplotype was 1986
13 \pm 536 ppm for the A SNP (X76713.5_620) - 3 bp insertion (bx1_1.9_143), 2890 \pm 239 ppm for G SNP
14 (X76713.5_620) - 3 bp insertion (bx1_1.9_143) , 1403 \pm 283 ppm for A SNP (X76713.5_620)- 3 bp deletion
15 (bx1_1.9_143), and 2128 \pm 317 ppm for G SNP (X76713.5_620)- 3 bp deletion (bx1_1.9_143). Among the
16 parental inbreds of the RILs families, B73, M37W, MS71, Oh43, and Tx303 carried the genetic variants at those
17 points favourable for DIMBOA-(T) accumulation (G at X76713.5_620 and 3 bp insertion at bx1_1.9_143); while
18 CML322, CML52, IL14H, and NC350 carried the unfavourable ones (A at X76713.5_620 and 3 bp deletion at
19 bx1_1.9_143).

20 In general, the level of linkage disequilibrium (LD) within the *bx1* gene rapidly decayed with distance (Fig.
21 2). However, LD coefficients (r^2) between the significant polymorphisms included in the ANOVA model,
22 X76713.5_620 or bx1_1.9-143, and other polymorphisms were > 0.2 up to a distance between polymorphisms of
23 approximately 600 bp (Fig. 1). All significant polymorphisms were in significant LD ($p < 0.01$) with X76713.5_620
24 and/or bx1_1.9-143, except two polymorphisms located at the beginning of *bx1* and the INDEL located at
25 X76713.5_40 (data not shown). The result that the initially significant polymorphism in *bx2* did not remained
26 significant in the multilocus model indicates that the initial significance is due to residual LD with the
27 polymorphisms in *bx1*.

1 Discussion

2

3 Heritabilities for DIMBOA levels were high in both RIL families tested in two years and differences between inbred
4 parents were significant for most traits. Therefore, QTLs with appreciable effect on DIMBOA content are expected
5 to be found. In the B73 × CML322 family 47.1 to 63.4 % of genetic variability for DIMBOA, DIMBOA-Glc and
6 DIMBOA-(T) among RILs could be explained by the detected QTLs. This is in contrast to only 4.5 to 21.2 % of
7 variability for RILs derived from B73 × I114H family. The B73 × CML322 family contrasts a moderate DIMBOA
8 parent with a low DIMBOA parent while the B73 × I114H family contrasts a moderate DIMBOA parent with a
9 slightly higher DIMBOA parent. These results suggest that undetected QTLs with small effects could be
10 responsible for unexplained differences for DIMBOA, DIMBOA-Glc, and DIMBOA-(T) contents in some
11 backgrounds such as the B73 × I114H family. Therefore, larger population sizes may be necessary to increase the
12 detection power and uncover where those genes lie. The NAM population could provide that increase in power if
13 those genes with minor effect are segregating in multiple RIL families.

14 The use of two environments for evaluations and cross validation allowed tests of the consistency of the
15 results (Bohn et al. 2001). Cross validations provide more reliable estimates of R^2_{adj} and \hat{a} by avoiding bias causing
16 model selection (Beavis 1998). Most QTLs found in both populations were reliable because the additive effects
17 computed with the CV/G data were significant and similar to those computed with the full data set. In addition,
18 most QTLs were found in more than 75% of CV/G runs. Therefore, the precision of QTL positions is adequate to
19 use markers close to them in MAS-assisted selection (Utz et al. 2000).

20 As QTLs for DIMBOA-(T) were, in general, the sum of QTLs detected for DIMBOA and DIMBOA-
21 Glc, we will focus discussion on DIMBOA-(T) content. Although the additive effects for the QTL were higher
22 among RILs derived from B73 × CML322 and accounted for a much higher percentage of genetic variability, two
23 QTLs, located in chromosomes 1 and 4, were involved in the DIMBOA pathway in both populations. The QTL on
24 chromosome 4 was located in both populations on the short arm of chromosome 4 where the *bx1*, *bx2*, *bx3*, *bx4*,
25 *bx5*, *bx6*, and *bx8* genes are located (<http://www.maizesequence.org/index.html>). Therefore, polymorphisms for
26 one or more of these structural genes of the DIMBOA pathway (Jonczyk et al. 2008) could be responsible for most
27 of the variance among (B73 × CML322) RILs and for almost 10 % of phenotypic variability among (B73 × I114H)
28 RILs. Bohn et al. (2001), Jampatong et al. (2002), and Cardinal et al. (2006) have reported QTLs for leaf-feeding
29 damage by lepidopterous species in the same genomic area, on chromosome 4 (bin 4.01). No significant QTLs were
30 found in the proximity of genes *bx7* (contig 160) and *bx9* (contig 37). In addition to the region of structural genes,
31 the involvement of other genomic regions (bins 1.03-1.04, 3.08, 6.01, 7.02, and 8.06) may reveal the position of

1 unknown regulatory genes (McMullen et al. 2009b). Regions in chromosomes 1 (1.04), 3 (3.08), 6 (6.01), and 7
2 (7.02), and 8 (8.06) had already been identified as involved in resistance to leaf feeding by *Diatraea grandiosella* and
3 *Ostrinia nubilalis* (Bohn et al. 1997; Groh et al. 1998; Jampatong et al. 2002; Brooks et al. 2005; 2007; Cardinal et al.
4 2006). Therefore, there is a convergence of loci for DIMBOA levels with loci detected in insect resistance QTL
5 experiments.

6 To extend the results from the two families of RIL to a broader range of germplasm, to determine the
7 degree of shared QTL across parents, and to test for allelic series at QTL for DIMBOA, eight families from the
8 NAM population were evaluated in 2008. The values for DIMBOA-(T) of each RIL family and its inbred parents
9 only differed for crosses B73 × CML322, B73 × CML52, M73 × MS71, and B73 × NC350. In general, the mean of
10 RIL families did not approximate the mid parent value for DIMBOA-(T) content, but exhibited DIMBOA levels
11 closer to the high or low parent or even showed less amount for DIMBOA-(T) content than both parents. These
12 data suggest that epistatic effects may play an important role in the inheritance of DIMBOA-(T) content. However,
13 a model including only additive effects explained approximately 34 % of phenotypic variability within RIL families
14 for DIMBOA-(T). As in the individual family analysis, the largest QTL in the across family analysis was located on
15 chromosome 4. The position of the significant marker, PHM1184, is on BAC contig 155, and co-localises with the
16 majority of structural genes of the DIMBOA pathway (www.maizegenome.org). In addition, seven other genomic
17 regions (located in chromosomes 1, 2, 4, 5, 6, and 8) explained as much variability as likely explained by
18 polymorphisms at structural genes. Previous supporting evidence of the potential involvement of some of these
19 genomic regions in DIMBOA synthesis comes from studies of QTLs for resistance to the first generation of *Ostrinia*
20 *nubilalis* (Jampatong et al. 2002; Cardinal et al. 2006) since resistance to the first generation of this insect in temperate
21 material is largely associated to midwhorl DIMBOA levels (Barry et al. 1994). Agreeing with those previous studies,
22 we have found QTLs for DIMBOA-(T) at bins 4.01, 4.04, 6.01, and 8.06. However, marker polymorphisms at bins
23 1.04, 2.04, 5.07, and 6.05 were not previously associated with phenotypic variation for resistance to the first
24 generation of *Ostrinia nubilalis*. The lack of prior report of these QTL for first generation resistance could be due to
25 either a low effect level of these loci or genetic heterogeneity as positive alleles at these loci only occurred in a subset
26 of the RIL families. All QTLs for DIMBOA content, except those located at 2.04, 4.04 and 5.07, were in regions
27 where QTL for resistance to leaf-feeding by *Diatraea grandiosella* and *D. saccharalis* were previously detected (Groh et
28 al. 1998, Khairallah et al. 1998; Bohn et al. 2001; Brooks et al. 2005; 2007).

29 Most QTLs detected when computing QTL analyses of individual RIL families were also included in the fit
30 model for the eight families, except those QTLs that explained less than 10% of variability within unique RIL
31 families or were located on the same chromosome as another detected QTL (data not shown). Therefore, the

1 procedure of searching for QTLs across individual RILs families could miss those QTLs with significant effects in
2 unique families, mostly when they are located in close proximity of other QTLs with significant effects across
3 populations. Yu et al. (2008) estimated the average power of NAM under different trait complexity schemes using
4 different number of RILs and determined that the average power could be approximately 0.6 when the number of
5 QTLs was 20, $h^2 = 0.7$, and the number of RILs = 1500. Consequently, it is not surprising that QTLs with low or
6 moderate additive effects in unique populations are not identified using NAM analysis, but the decrease in power of
7 detection when performing NAM analysis compared to conventional linkage analysis seemed to be low. Moreover,
8 due to the increased resolution, additive effects for markers detected by the NAM approach were highly significant
9 ($p < 0.001$) for particular RIL families in which no QTLs were found near to the marker using conventional linkage
10 mapping (data not shown).

11 The tropical lines used in this study, CML322, CML52, and NC350, supplied alleles for the QTL at 4.01
12 with significant negative effects for DIMBOA-(T) concentration. This would explain why most studies conducted
13 to locate QTLs for resistance to leaf feeding by native American lepidopterous insects among tropical and
14 subtropical inbreds did not report QTLs close to the *bx* region (Bohn et al. 2001; Brooks et al. 2005; 2007). This
15 result also agrees with the concept that different mechanisms from DIMBOA antibiosis are present in resistant
16 tropical maize. Bohn et al. (2001) found QTLs for resistance to leaf feeding by *Diatraea grandiosella* at chromosome
17 positions 4.01 and 6.01, where QTLs for DIMBOA-(T) have been located, but the favourable alleles for resistance
18 to the pest came from the susceptible tropical inbred (Ki3), and not from the resistance source.

19 The additive effects of the marker PZA00635 (at bin position 2.04) were significant in four RIL families,
20 this marker is located in contig 80, close to the position of a gene that codes for a specific glucosidase (*β -glu2* in
21 contig 84). It is unclear how variability for a specific glucosidase for DIMBOA-Glc, whose activity renders
22 DIMBOA from DIMBOA-Glc (Morant et al. 2008), could affect variability for DIMBOA-(T) content.

23 For markers in chromosomes 1 at bin 1.04, 4 at bin 4.01, and 6 at bins 6.01 and 6.05, there were at least
24 three different alleles because non-B73 parents supplied alleles with increased and decreased additive effects for
25 DIMBOA-(T) content compared to B73. Therefore, although no more than two alleles were segregating in each
26 RIL family, allowing a high detection power, the additive effects of more than two allelic variants per loci could be
27 simultaneously estimated using the multiple family approach of NAM.

28 Previous studies showed that DIMBOA concentration decreases with plant age (Morse et al. 1991; Barry et
29 al. 1994; Cambier et al. 2000). We demonstrated a significant, negative association between DIMBOA-(T) content
30 and plant growth measured as number of leaves per plant. Therefore we reasoned that factors regulating growth
31 could also appear as QTL for DIMBOA synthesis. Three QTLs involved in differences for number of leaves were

1 found and markers linked to those QTLs explained approximately the 9% of phenotypic variation for number of
2 leaves, but none of these markers were co-localized to markers associated with variation for DIMBOA-(T) content.
3 Therefore, none of the QTL identified for DIMBOA synthesis are genetically correlated to growth loci. The
4 apparent negative correlation of leaf number with DIMBOA-(T) must involve small genetic effects not detected as
5 significant QTL.

6 As position of the largest QTL co-localizes with the majority of structural genes of the DIMBOA pathway,
7 and *bx1* is the committal step into the DIMBOA pathway, it is the prime candidate for affecting total DIMBOA
8 accumulation. Therefore association analysis was used to determine if genetic variation at *bx1* affects variation of
9 DIMBOA-(T) content. General (GLM) and Mixed Linear Models (MLM) revealed associations between variability
10 for DIMBOA-(T) content and sequence polymorphisms at *bx1* locus. After adjusting for population structure, a
11 model including two polymorphisms in *bx1* explained 12% of phenotypic variation in a population of 282 diverse
12 lines. This is close to the 15% effect predicted by the GLMSELECT analysis for the chromosome 4 QTL across
13 eight families of RILs. However, these polymorphisms could not explain genetic effects for DIMBOA-(T) content
14 of the closest marker (PHM1184) to the major QTL detected in chromosome 4. For example, inbreds CML322 and
15 IL14H carried the same genetic variants at both polymorphisms, but among RILs families obtained from crosses to
16 B73, the allele at PHM1184 from CML322 had a negative additive effect for DIMBOA-(T) content, while the allele
17 from IL14H had a positive effect. Therefore, the high percentage of variation explained by the model including the
18 two polymorphisms at exons 5 and 6 in *bx1* and their significant LD with other significantly associated
19 polymorphisms in *bx1* and *bx2* suggests that genetic variation for *bx1* is particularly important for determining
20 DIMBOA content in a diverse panel of maize inbred lines. The rapid decay of LD and the fact that no
21 polymorphisms in *bx2* were significant in the presence of a significant *bx1* polymorphism suggest that the main
22 causal polymorphisms are within or near the *bx1* gene and alter DIMBOA content by affecting BX1 level.
23 However, the specific causal polymorphism or polymorphisms responsible for the QTL detected in the region 4.01
24 were not identified, either because those polymorphisms were not sequenced (*bx1* was not entirely sequenced),
25 causal polymorphism is outside the coding region, identity is masked by linkage disequilibrium, adjustments for
26 population structure reduce significance of causal polymorphisms or multiple causal polymorphisms affecting *bx1*
27 segregate among inbred lines. In characterizing QTL for flowering time in maize Buckler et al. (2009) demonstrated
28 that common QTL with uncommon, multiple alleles is the norm. It may be that *bx1* from IL14H has a distinct
29 causal polymorphism increasing DIMBOA levels from the causal polymorphisms in LD with the significant SNP
30 detected in this study.

31

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2

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9 Names of products are necessary to report factually on available data; however, neither the USDA nor any other
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11 approval of the product or the exclusion of others that may also be suitable.

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6

Supplemental Table 1. Characteristics of the amplicons covering *bx6* and evidence-genes for *bx1* (AC200309.3:82911-85155, GRMZM2G085381) from 36 bp downstream of the 5' end to 148 bp upstream of 3' end, *bx2* (AC200309.3:87362-89517, GRMZM2G085661) from 55bp upstream of the 5' end to 560 downstream of 5' end, *bx3* (AC193441.3: 7574-10352, GRMZM2G167549) from 1755 bp downstream of the 5' end to 371 upstream of 3' end, *bx4* (AC213878.3: 111418-114239, GRMZM2G172491) from 1140 to 1389 bp downstream of the 5' end and from 1752 bp downstream of the 5' end to 484 upstream of 3' end, *bx5* (AC213878.3: 49953-52529, GRMZM2G063756) from 365 bp downstream of the 5' end to 1018 bp downstream of the 5' and from 1699 bp downstream of the 5' end to 540 bp upstream of the 3' end, and *bx8* (AC200309.3:36796-38707, GRMZM2G085054) from 1311 downstream of 5' to 52 upstream of 3' end.

Initial sequence	Gene	Primer name	Primer sequence	Start (bp)	Tm (°C)	Size (bp)
AC200309.3:82911-85155 ¹	<i>bx1</i>	BX1_1.10F	AACGGACAGGTTGTTGCACAG	36	63.42	713
		BX1_1.10R	GCTTGTAGTAGGAGAGGAGCACCA	748	63.25	
X767713 ²	<i>bx1</i>	X76713.2F	TGGTGCTCCTCTCCTACTACAAGC	2477	63.25	606
		X76713.2R	TCACCTTCTTAACCTCCTGGATGA	3082	63.3	
X767713 ²	<i>bx1</i>	X76713.5F	GAGTGAAGCCAAGAACAACAACCT	2688	63.04	657

		X76713.5R	CATACTCCTCCAGCCTCCTCAG	3344	62.54	
AC200309.3:82911-85155 ¹	<i>bx1</i>	BX1_1.9F	CCTGAGGAGGCTGGAGGAGTAT	1574	63.19	523
		BX1_1.9R	AAGGCATTTAACAGGAAGGAGTACG	2096	62.93	
AC200309.3: 87241-89517 ¹	<i>bx2</i>	BX2_2.8F	AAATAAACGCTCCAAAGAAAGCAA	67	62.38	615
		BX2_2.8R	GAGGAGAAGACGTGGTTCGTAGGT	681	63.12	
Y11404 ²	<i>bx3</i>	Y11404.3F	TGGTGGGTAAGTCCAGCTACTGTT	1505	63.25	683
		Y11404.3R	GAACCTCCCAATCAAAAATGGTACA	2187	63.21	
X81828 ²	<i>bx4</i>	X81828.4F	CCAAATGGAAGCAGCACAGG	2275	63.98	698
		X81828.4R	AAGCTCGTTGAAGAGGCTGTC	2971	63.26	

X81828 ²	<i>bx4</i>	X81828.5F	GACCTGTTCCCTCCGGATCATCT	2898	63.87	617
		X81828.5R	AGAACTCCTCGGCCTTGTC	3514	62.98	
AC213878.1:115151-115887 ¹	<i>bx5</i>	BX5_5.3F	CGGAAGGAGTAGACCTTCTTGTTG	225	62,6	507
		BX5_5.3R	CCAGGCAGCCTACGAGTACCT	731	62,92	
Y11403 ²	<i>bx5</i>	Y11403.4F	TCCTCTCCAGATACGAGGAGTACG	3215	63.11	700
		Y11403.4R	CAGTCGAAATGGTACATGAGGTTG	3914	62.83	
Y11403 ²	<i>bx5</i>	Y11403.5F	CCAGGCAGCCTACGAGTACCT	2211	62.92	608
		Y11403.5R	TTCATGCAGGATTATTCCITTAATTTTC	2818	62.68	

AC200309.3: 36796-38707 ¹	<i>bx8</i>	BX8_8.7F	AGCTGGAGAGAGGGGAGATCAA	1311	63.86	549
		BX8_8.7R	TGTGCACATGATACTTCGCCTTTA	1859	63.82	

¹ Position at the B73 sequence database.

² GenBank sequence's name

Table 1. LSMeans of the parents¹ and RILs, RIL range and heritability (H) for DIMBOA, DIMBOA–Glc, and DIMBOA-(T) content (ppm) evaluated in 2007 and 2008.

		B73 x CML322			B73 x IL14H			
Year		Genotype	DIMBOA	DIMBOA-Glc	DIMBOA-(T)c	DIMBOA	DIMBOA-Glc	DIMBOA-(T)
2007	Mean	P1	1211.39 a	1800.66 a	3012.05 a	613.21 b	2482.40 b	3095.61 b
		P2	36.87 b	1013.38 b	1050.24 b	1527.12 a	4310.23 a	5837.35 a
		RILs	583.81	1544.43	2128.23	1385.59	3107.88	4493.47
2008	Mean	P1	602.97 a	1644.00 a	2246.97 a	410.16 b	1490.43 a	1900.59 a
		P2	603.88 a	1699.75 a	2303.63 a	796.23 a	1451.71 a	2247.94 a
		RILs	229.21	985.68	1213.97	594.30	1680.83	2261.00
Across	Mean	P1	907.19 a	1722.33 a	2629.51 a	511.69 b	1986.41 b	2498.10 b
		P2	320.38 b	1356.56 a	1676.93 b	1161.68 a	2880.97 a	4042.64 a
		RILs	405.21	1259.53	1664.25	999.99	2378.21	3371.34
	Range	RILs	0-2240	0-4120	0-6112	0-4343	0-6918	365-10927
	H (%)	RILs	63.2	82.4	87.4	82.9	68.7	79.8

¹ Parent means followed by the same letter did not differ at the 0.05 probability level.

Table 2. Summary of QTLs affecting DIMBOA (D), DIMBOA–Glc (D_g), and DIMBOA-(I) (D_t) across years in a maize RIL family derived from the cross B73 x CML322, with their respective additive effects determined using the whole data set (\hat{a}) or 200 five-fold cross validation runs ($\hat{a}_{TS,ES}$)

Trait	Chr ¹	Position	Left				Cross					
			Confidence interval	LOD score	flanking marker	\hat{a}^2	$R^2_{adj}{}^3$	$\% \sigma_g^2{}^4$	validation $\hat{a}_{TS,ES}{}^5$			
		cM	cM						(10;90)	Freq		
									Median	Percentile	%	$R^2_{adj}{}^6$
D	17	78	74-82	10.03	L00776	-143.9±21.9 ⁹	19.6		-131.3	(-108.4; -154.8)	97.3	
	4 ⁷	0	0-2	17.13	L00959	-190.7±21.1 ⁹	31.5		-209.1	(-183.8; -236.7)	99.9	
	8	102	94-110	3.19	L00489	-66.5±21.1 ⁹	5.3		-84.3	(-72.3; -102.9)	42.2	
							43.4	47.1				30.7
D _g	1 ⁸	78	70-82	3.07	L00776	-188.4±40.4 ⁹	11.1		-165.2	(-141.3; -210.7)	47.5	
	3	118	112-122	5.46	L00286c	137.0±38.8	6.6		251.5	(158.2; 330.7)	92.7	
	4 ⁸	0	0-2	18.04	L00959	-400.4±38.9 ⁹	37.4		-376.2	(-330.6; -421.9)	100.0	
	8 ⁸	82	76-86	5.21	L00534	-208.5±38.8 ⁹	14.1		-193.2	(-154.1; -240.8)	80.5	
							48.9	63.4				40.0
D _t	1 ⁸	78	74-82	6.69	L00776	-334.9±53.1 ⁹	18.4		-261.5	(-211.5; -325.7)	97.2	
	3	118	114-126	3.43	L00286c	174.7±51.7	6.2		242.4	(186.8; 382.6)	79.0	
	4 ⁸	4	0-2	23.57	L00959	-585.7±51.3 ⁹	42.5		-592.0	(-532.1; -660.2)	100.0	

7	7	60-68	4.71	L00545	149.2±51.6 ⁹	4.5	363.9	(257.3; 496.8)	82.1
8 ⁸	100	94-108	7.40	L00489	-289.4±54.8 ⁹	13.7	-229.8	(-192.6; -290.3)	62.0
						51.9	55.3		49.1

¹ Chromosome where the QTL is located. ² Mean additive effect ± standard deviation based on whole data, negative effects mean that the allele for higher amount came from B73. ³ Proportion of phenotypic variance explained. ⁴ Proportion of the genotypic variance explained by detected QTL after adjusting for QTL x Environment interactions. ⁵ Median, percentiles, and frequency of QTL detection were calculated based on 200 fivefold CV/G runs. ⁶ Proportion of phenotypic variance explained by detected QTLs calculated in 200 cross validations runs. ⁷ QTL x Environment effect was significant. ⁸ QTL x Environment effect was not significant. ⁹ Additive effects were significant in each individual environment.

Table 3. Summary of QTLs affecting DIMBOA (D), DIMBOA–Glc (D_g), and DIMBOA-(T) (D_t) across years in a maize RIL family derived from the cross B73 x IL14H, with their respective additive effects determined using the whole data set (\hat{a}) or 200 five-fold cross validation runs ($\hat{a}_{TS,ES}$)

Trait	Chr ¹	Position	Confidence interval	LOD score	Left flanking marker	\hat{a}^2	$R^2_{adj}{}^3$	$\% \sigma_g^2{}^4$	Cross validation			
									$\hat{a}_{TS,ES}{}^5$		Freq	
									(10;90)			
									Median	Percentile	%	$R^2_{adj}{}^6$
D	1 ⁸	144	140-148	5.31	L01175	215.5±48.2 ⁹	10.1		219.3	(187.4; 274.0)	86.2	
	6 ⁸	6	0-16	4.70	L00743	195.7±51.3 ⁹	7.6		228.2	(192.8; 270.6)	86.3	
	6 ⁸	82	78-86	3.25	L00448	175.7±47.2 ⁹	7.3		201.4	(174.3; 363.7)	42.9	
							19.1	21.2				6.6
D _g	4 ⁸	10	4-16	4.11	L00074	397.8±95.4 ⁹	8.8		409.8	(350.9; 487.5)	67.3	
							7.8	4.5				2.7
D _t	1	62	50-66	3.06	L00116	-369.2±130.0 ⁹	4.3		-513.4	(-452.8; -605.9)	26.7	
	4 ⁸	10	6-16	5.05	L00074	554.0±128.1 ⁹	9.4		578.1	(491.1; 678.5)	76.5	
	6 ⁸	6	0-16	4.52	L00743	465.1±130.7 ⁹	6.6		562.9	(481.8; 671.9)	80.4	
							16.1	5.4				4.5

¹ Chromosome where the QTL is located. ² Mean additive effect ± standard deviation based on whole data, negative effects mean that the allele for higher amount came from B73. ³ Proportion of phenotypic variance explained. ⁴ Proportion of the genotypic variance explained by detected QTL after adjusting for QTL x Environment interactions. ⁵ Median, percentiles, and frequency of QTL detection were calculated based on 200 fivefold CV/G runs. ⁶ Proportion of phenotypic

variance explained by detected QTLs calculated in 200 cross validations runs. ⁷ QTL x Environment effect was significant. ⁸ QTL x Environment effect was not significant. ⁹ Additive effects were significant in each individual environment.

Supplemental Table 2. LSMeans¹ of the inbred parents and the RIL family derived from them for DIMBOA-(I) evaluated in 2008 and the correlation coefficients between DIMBOA-(I) content and number of leaves among recombinant inbred lines.

	B73 x CML322	B73 x CML52	B73 x IL14H	B73 x M37W	B73 x MS71	B73 x NC350	B73 x Oh43	B73 x Tx303
Means								
Female parent	2246.97 a	3635.09 a	1900.59 a	3742.05 a	2873.46 b	3249.21 a	3792.96 a	1601.65 a
Male parent	2303.63 a	1216.99 b	2247.93 a	4715.89 a	5342.08 a	171.64 c	4881.33 a	1321.77 a
RILs	1213.98 b	3428.77 a	2261.90 a	4467.52 a	3782.48 b	1763.52 b	3831.86 a	1573.29 a
Correlation								
coefficients	-0.01	-0.21**	-0.06	-0.18*	-0.16*	-0.06	-0.23**	-0.09

¹ Means followed by the same letter did not differ at the 0.05 probability level.

*, ** Significant at 0.05 and 0.01 probability levels, respectively.

Table 4. Position, and effects of markers identified as significant for DIMBOA-(T) (ppm) content in the GLM model. Significant effects are in bold.

Marker	Chrom ¹	Position ²	Contig	Parameter ³	CML322	CML52	IL14H	M37W	MS71	NC350	Oh43	Tx303	R ²
DIMBOA-(T)													
PZA03189	1	73.3	ctg14	Effect	-213.9⁵	-328.0⁵	-163.6	-227.3	295.7	-635.4⁵	-235.0	-98.5	2.80 ⁴
				<i>p</i>	0.049	0.002	0.169	0.048	0.004	<0.001	0.031	0.356	
PZA00635	2	73.5	ctg80	Effect	-55.1	-252.5	-85.0	-637.6⁵	-379.6⁵	-84.5	-222.2	-53.5	3.41
				<i>p</i>	0.609	0.024	0.454	<0.001	<0.001	0.430	0.033	0.616	
PHM1184	4	7.2	ctg155	Effect	-489.8⁵	-1061.9⁵	300.2	325.1	435.4⁵	-338.7	1042.8⁵	47.0	15.74
				<i>p</i>	<0.001	<0.001	0.020	0.001	<0.001	0.001	<0.001	0.660	
PZA02002	4	49.4	ctg163	Effect	-39.5	49.5	99.1	498.9	420.1	153.5	205.8	-36.3	2.44
				<i>p</i>	0.725	0.641	0.496	<0.001	<0.001	0.156	0.053	0.731	
PZA00980	5	114.3	ctg251	Effect	-6.8	333.6	200.8	675.2⁵	122.5	189.0	320.0	195.3	3.34
				<i>p</i>	0.963	0.006	0.080	<0.001	0.263	0.080	0.002	0.061	
PZA01527	6	8.8	ctg265	Effect	97.2	332.4	226.7	22.4	487.1⁵	27.8	-410.1	122.5	2.40
				<i>p</i>	0.368	0.001	0.056	0.828	<0.001	0.801	<0.001	0.245	
PZA00473	6	45.9	-	Effect	127.6	-215.1⁵	262.5	268.8	255.2	-134.2	427.7	23.0	1.61
				<i>p</i>	0.253	0.036	0.026	0.012	0.028	0.242	<0.001	0.844	
PZA02746	8	94.1	ctg362	Effect	-262.9⁵	-108.5	-262.9	-389.5	-137.4	-409.5	-262.1	-24.2	2.26
				<i>p</i>	0.017	0.317	0.023	<0.001	0.190	<0.001	0.014	0.827	

¹Chromosome where the QTL is located. ² Position of the marker in cM. ³Mean additive effect of the marker in each RIL family with its corresponding probability

level, positive effects negative effects mean that the allele for higher amount came from B73 ⁴Proportion of intra-population phenotypic variance explained. ⁵ The

QTL identified by individual analysis of the specific RIL family was less than 5 cM apart from the marker identified as associated to DIMBOA-(T) content across populations.

Supplemental table 3 . Genotypes for 177 inbred lines at each SNP and INDEL in *bx1* associated significantly to DIMBOA content

Amplicon name	bx1_1.10																X76713.2												X76713.5												bx1_1.9			Haplotype number
	Position (bp)	337	351	358	381	427	238	364	395	416	425	445	448	449	162	175	193	214	223	233	237	244	247	248	480	620	634	634	635	652	653	685	686	687	687	688	690	692	693	731	12	22	143	
CML332	0	0	0	C	0	0	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	0	3	1	
NC300	0	0	0	C	0	0	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	0	3	1	
NC340	0	0	0	C	0	0	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	0	3	1	
NC296	0	0	0	C	0	1	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	0	3	2	
NC336	0	0	0	C	0	1	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	0	3	2	
VaW6	0	0	0	C	0	1	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	0	3	2	
CML314	0	0	10	C	0	0	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	0	3	3	
CO255	0	0	10	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	3	0	3	4	
F7	0	0	10	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	3	0	3	4	
NC264	.	17	17	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	3	0	3	5	
CML158Q	0	0	10	C	0	1	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	G	-	5	.	0	0	C	G	G	0	0	A	T	A	-	0	3	3	6	
I205	0	0	0	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	2	3	3	7	
MS153	0	0	0	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	2	3	3	7	
CML10	0	0	0	C	0	0	C	C	5	C	A	A	6	C	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	C	G	-	3	.	G	T	A	A	3	3	3	8	
L578	0	0	0	C	0	0	C	T	5	C	G	G	26	C	0	T	5	C	C	C	179	G	5	1	G	-	5	.	0	0	C	G	G	0	0	A	T	A	-	3	3	3	9	
Tx601	0	0	0	C	0	0	.	T	5	C	G	G	38	C	0	T	5	C	C	G	217	-	.	0	A	-	1	.	0	0	T	A	A	2	.	.	C	G	.	3	3	3	10	
Tzi11	0	0	0	C	0	0	C	T	5	C	G	G	38	C	0	T	5	C	C	G	217	-	.	0	A	-	1	.	0	0	T	A	A	2	2	G	C	G	.	3	3	3	10	
CML157Q	0	0	0	C	0	0	C	T	5	C	G	G	38	C	0	T	5	C	C	G	217	-	.	0	A	-	1	.	0	0	T	A	A	2	2	G	C	G	G	3	3	3	10	
NC362	0	0	0	C	0	0	C	T	5	C	G	G	38	C	0	T	5	C	C	G	217	-	.	0	A	-	1	.	0	0	T	A	A	2	2	G	C	G	G	3	3	3	10	
NC298	0	0	0	C	0	0	C	T	5	C	G	G	38	C	0	T	5	C	C	G	217	-	.	0	A	-	1	.	0	0	T	A	A	2	.	.	C	G	.	3	3	3	10	
NC348	0	0	0	C	0	1	C	T	.	G	G	G	38	C	0	T	5	C	C	G	217	-	.	0	A	-	1	.	0	0	T	A	A	2	2	G	C	G	G	3	3	3	10	
CML38	0	0	0	C	0	0	G	.	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	11	
CML328	0	0	0	C	0	0	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	11	
Oh603	0	0	0	C	0	0	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	11	
CML52	0	0	0	C	0	0	G	C	5	C	G	G	27	G	0	C	5	C	C	G	179	G	0	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	12	
CML258	0	0	0	C	0	1	C	C	5	C	A	A	26	C	0	C	5	C	C	G	179	A	5	1	A	T	0	0	3	3	C	G	-	3	.	G	T	A	G	3	3	3	13	
CM105	0	0	0	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	.	3	3	3	14	
A632	0	0	0	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	3	3	3	14	
A635	0	0	0	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	3	3	3	14	
B14A	0	0	0	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	3	3	3	14	
B84	0	0	0	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	3	3	3	14	
CM174	0	0	0	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	3	3	3	14	
CMV3	0	0	0	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	3	3	3	14	
DE2	0	0	0	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	3	3	3	14	
H91	0	0	0	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	3	3	3	14	
Os420	0	0	0	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	3	3	3	14	
DE3	0	.	0	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	3	3	3	14	
A272	0	0	0	C	0	1	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	15	
A441-5	0	0	0	C	0	1	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	15	
B75	0	0	0	C	0	1	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	15	
CML277	0	0	0	C	0	1	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	15	
CML311	0	0	0	C	0	1	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	15	
CML323	0	0	0	C	0	1	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	15	
CO125	0	0	0	C	0	1	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	15	
T234	0	0	0	C	0	1	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	15	
Tzi10	0	0	0	C	0	1	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	15	
W153R	0	0	0	C	0	1	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3</														

Mo44	0	0	10	C	0	0	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	19
NC260	0	0	10	C	0	0	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	19
Oh43E	0	0	10	C	0	0	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	19
Va14	0	0	10	C	0	0	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	19
3811	0	0	10	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	3	3	3	20
Pa91	0	0	10	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	3	3	3	20
CML281	0	0	10	C	0	1	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	21
T8	0	0	10	C	0	1	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	21
TzI9	0	0	10	C	0	1	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	21
Va35	0	0	10	C	0	1	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	21
Va59	0	0	10	C	0	1	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	21
CI3A	4	17	17	-	5	1	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	22
MEF156552	.	17	17	C	0	0	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	23
CI1872	.	17	17	C	0	1	C	T	0	G	G	G	38	C	3	T	.	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	3	3	3	24
A659	.	17	17	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	3	3	3	24
B52	.	17	17	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	3	3	3	24
C49A	.	17	17	C	0	1	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	25
L317	.	17	17	C	0	1	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	25
MT42	.	17	17	C	0	1	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	25
OH7B	.	17	17	C	0	1	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	25
Pa875	.	17	17	C	0	1	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	25
Va99	.	17	17	C	0	1	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	25
SA24	.	17	17	C	0	1	C	T	5	C	G	G	38	C	0	T	5	C	C	G	217	-	.	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	26
HP301	.	17	17	C	0	1	C	T	5	C	G	G	38	C	0	T	5	C	C	G	217	-	.	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	26
IDS69	.	17	17	C	0	1	C	T	5	C	G	G	38	C	0	T	5	C	C	G	217	-	.	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	26
Sg18	.	17	17	C	0	1	C	T	5	C	G	G	38	C	0	T	5	C	C	G	217	-	.	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	26
Mo24W	4	17	17	G	5	1	C	T	5	C	G	G	38	C	0	T	5	C	C	G	217	-	.	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	27
Ki14	4	.	17	G	5	1	C	T	5	G	G	G	37	C	0	T	5	G	C	G	217	-	.	1	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	3	3	3	27
CML220	0	0	0	C	0	0	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	6	3	3	28
B103	0	0	0	C	0	0	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	13	13	0	29
K4	0	0	0	C	0	0	G	C	5	C	A	-	27	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	13	13	0	30
WD	0	0	0	C	0	1	C	C	5	G	G	C	27	C	3	C	5	G	C	G	179	C	5	0	G	C	0	1	2	0	C	G	G	0	0	A	T	A	A	13	13	0	31
IA5125	0	0	0	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	13	13	0	32
IDS28	0	0	0	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	13	13	0	32
NC222	0	0	0	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	13	13	0	32
NC236	0	0	0	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	13	13	0	32
NC33	0	0	0	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	13	13	0	32
B97	0	0	0	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	2	0	C	G	G	0	0	A	T	A	A	13	13	0	32
II101	0	0	0	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	2	0	C	G	G	0	0	A	T	A	A	13	13	0	32
NC230	0	0	0	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	2	0	C	G	G	0	0	A	T	A	A	13	13	0	32
NC258	0	0	0	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	2	0	C	G	G	0	0	A	T	A	A	13	13	0	32
NC290A	0	0	0	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	2	0	C	G	G	0	0	A	T	A	A	13	13	0	32
NC360	0	0	0	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	2	0	C	G	G	0	0	A	T	A	A	13	13	0	32
NC366	0	0	0	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	2	0	C	G	G	0	0	A	T	A	A	13	13	0	32
T232	0	0	0	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	2	0	C	G	G	0	0	A	T	A	A	13	13	0	32
W117HT	0	0	0	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	2	0	C	G	G	0	0	A	T	A	A	13	13	0	32
Tzi25	0	0	0	C	0	1	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	13	13	0	33
A188	0	0	0	C	0	1	C	T	5	G	G	G	8	C	0	T	5	G	C	G	179	G	8	0	G	-	5	.	0	0	C	G	G	0	0	A	T	A	-	13	13	0	34
AB28A	0	0	0	C	0	1	C	T	5	G	G	G	8	C	0	T																											

B73	0	0	10	C	0	0	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	G	-	5	.	1	0	C	G	G	0	0	A	T	A	A	13	13	0	38
B73HTRHM	0	0	10	C	0	0	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	G	-	5	.	1	0	C	G	G	0	0	A	T	A	A	13	13	0	38
CML154Q	0	0	10	C	0	0	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	G	-	5	.	1	0	C	G	G	0	0	A	T	A	A	13	13	0	38
NC306	0	0	10	C	0	0	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	G	-	5	.	1	0	C	G	G	0	0	A	T	A	A	13	13	0	38
NC310	0	0	10	C	0	0	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	G	-	5	.	1	0	C	G	G	0	0	A	T	A	A	13	13	0	38
NC314	0	0	10	C	0	0	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	G	-	5	.	1	0	C	G	G	0	0	A	T	A	A	13	13	0	38
NC326	0	0	10	C	0	0	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	G	-	5	.	1	0	C	G	G	0	0	A	T	A	A	13	13	0	38
NC328	0	0	10	C	0	0	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	G	-	5	.	1	0	C	G	G	0	0	A	T	A	A	13	13	0	38
NC368	0	0	10	C	0	0	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	G	-	5	.	1	0	C	G	G	0	0	A	T	A	A	13	13	0	38
CI90C	0	0	10	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	2	0	C	G	G	0	0	A	T	A	A	13	13	0	39
Hy	0	0	10	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	2	0	C	G	G	0	0	A	T	A	A	13	13	0	39
M14	0	0	10	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	2	0	C	G	G	0	0	A	T	A	A	13	13	0	39
B68	0	0	10	C	0	1	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	13	13	0	40
A619	0	0	10	C	0	1	C	T	5	G	G	G	8	C	0	T	5	G	C	G	179	G	8	0	G	-	5	.	1	0	C	G	G	.	3	-	T	A	-	13	13	0	41
H99	0	0	10	C	0	1	C	T	5	G	G	G	8	C	0	T	5	G	C	G	179	G	8	0	G	-	5	.	1	0	C	G	G	.	3	-	T	A	-	13	13	0	41
Oh40B	0	0	10	C	0	1	C	T	5	G	G	G	8	C	0	T	5	G	C	G	179	G	8	0	G	-	5	.	1	0	C	G	G	.	3	-	T	A	-	13	13	0	41
Pa762	0	0	10	C	0	1	C	T	5	G	G	G	8	C	0	T	5	G	C	G	179	G	8	0	G	-	5	.	1	0	C	G	G	.	3	-	T	A	-	13	13	0	41
A214N	0	0	10	C	0	1	G	C	5	C	A	A	26	G	0	C	5	C	C	G	179	A	5	1	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	13	13	0	42
3316	4	17	17	-	5	1	C	T	5	G	G	G	8	C	0	T	5	G	C	G	179	G	8	0	G	-	5	.	1	0	C	G	G	.	3	-	T	A	-	13	13	0	43
H95	.	17	17	C	0	0	G	C	5	C	A	A	6	G	0	C	5	C	C	G	179	A	6	0	A	T	0	0	3	3	T	A	A	2	2	G	C	G	G	13	13	0	44
CI31A	.	17	17	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	13	13	0	45
GA209	.	17	17	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	13	13	0	45
CML103	.	17	17	C	0	1	.	T	.	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	2	0	C	G	G	0	0	A	T	A	A	13	13	0	46
W64A	.	17	17	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	2	0	C	G	G	0	0	A	T	A	.	13	13	0	46
CO106	.	17	17	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	2	0	C	G	G	0	0	A	T	A	A	13	13	0	46
F6	.	17	17	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	2	0	C	G	G	0	0	A	T	A	A	13	13	0	46
MS1334	.	17	17	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	2	0	C	G	G	0	0	A	T	A	A	13	13	0	46
MS71	.	17	17	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	2	0	C	G	G	0	0	A	T	A	A	13	13	0	46
ND246	.	17	17	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	2	0	C	G	G	0	0	A	T	A	A	13	13	0	46
R177	.	17	17	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	2	0	C	G	G	0	0	A	T	A	A	13	13	0	46
R4	.	17	17	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	2	0	C	G	G	0	0	A	T	A	A	13	13	0	46
SC357	.	17	17	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	2	0	C	G	G	0	0	A	T	A	A	13	13	0	46
Va102	.	17	17	C	0	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	2	0	C	G	G	0	0	A	T	A	A	13	13	0	46
NC238	4	.	17	G	5	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	2	0	C	G	G	0	0	A	T	A	A	13	13	0	46
B115	4	17	17	G	5	1	C	T	0	G	G	G	38	.	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	13	13	0	47
Mo17	4	17	17	G	5	1	C	T	0	G	G	G	38	C	3	T	.	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	13	13	0	47
Mp339	4	17	17	G	5	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	.	13	13	0	47
A682	4	17	17	G	5	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	13	13	0	47
CI91B	4	17	17	G	5	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	13	13	0	47
E2558W	4	17	17	G	5	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	13	13	0	47
M162W	4	17	17	G	5	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	13	13	0	47
Mo45	4	17	17	G	5	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	13	13	0	47
Mo46	4	17	17	G	5	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	13	13	0	47
Mo47	4	17	17	G	5	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	13	13	0	47
B76	.	17	17	C	.	1	C	T	0	G	G	G	38	C	3	T	0	G	A	C	179	G	32	1	G	C	0	1	1	0	C	G	G	0	0	A	T	A	A	13	13	0	47
F2834T	4	17	17	G	5	1	C	T	5	G	G	G	8	C	0	T	5	G	C	G	179	G	8	0	G	-	5	.	0	.	C	G	G	0	0	A	T	A	-	13	13	0	48
D940Y	4	17	17	G	5	1	C	T	5	G	G	G	8	C	0	T	5	G	C	G	179	G	8	0	G	-	5	.	0	0	C	G	G	0	0	A	T	A	-	13	13	0	48
F44	4	17	17	G	5	1	C	T	5	G	G	G	8	C	0	T	5	G	C	G	179	G	8	0	G	-	5	.	0	0	C	G	G	0	0	A	T	A	-	13	13	0	48
B2	4	17	17	G	5																																						

Figures

Fig. 1 Association of DNA polymorphisms with DIMBOA-(T) content across genomic portions of genes *bx1* (82911-85155 bp) and *bx2* (87362-89517 bp). The positions correspond to BAC sequence AC200309.3. Blue diamonds indicate association with DIMBOA content. Level of statistical association for each SNP and INDEL is expressed as $-\text{Log}_{10}[P]$. Pink squares indicate r^2 LD scores for all marker pairs involving X76713.5_620 and yellow triangles r^2 LD scores for all marker pairs involving bx1_1.9_143.

Fig. 2 Linkage disequilibrium (LD) across *bx1* and part of *bx2* genes.



