Theor Appl Genet (2013) 126:2257–2266
DOI 10.1007/s00122-013-2132-7

ORIGINAL PAPER

QTL mapping of stalk bending strength in a recombinant inbred line maize population

Haixiao Hu · Wenxin Liu · Zhiyi Fu · Linda Homann · Frank Technow · Hongwu Wang · Chengliang Song · Shitu Li · Albrecht E. Melchinger · Shaojiang Chen

Received: 12 February 2013 / Accepted: 21 May 2013 / Published online: 5 June 2013

Abstract Stalk bending strength (SBS) is a reliable indicator for evaluating stalk lodging resistance of maize plants. Based on biomechanical considerations, the maximum load exerted to breaking ($F_{\max}$), the breaking moment ($M_{\max}$) and critical stress ($\sigma_{\max}$) are three important parameters to characterize SBS. We investigated the genetic architecture of SBS by phenotyping $F_{\max}$, $M_{\max}$ and $\sigma_{\max}$ of the fourth internode of maize plants in a population of 216 recombinant inbred lines derived from the cross B73 × Ce03005 evaluated in four environments. Heritability of $F_{\max}$, $M_{\max}$ and $\sigma_{\max}$ was 0.81, 0.79 and 0.75, respectively. $F_{\max}$ and $\sigma_{\max}$ were positively correlated with several other stalk characters. By using a linkage map with 129 SSR markers, we detected two, three and two quantitative trait loci (QTL) explaining 22.4, 26.1 and 17.2 % of the genotypic variance for $F_{\max}$, $M_{\max}$ and $\sigma_{\max}$, respectively. The QTL for $F_{\max}$, $M_{\max}$ and $\sigma_{\max}$ located in adjacent bins 5.02 and 5.03 as well as in bin 10.04 for $F_{\max}$ were detected with high frequencies in cross-validation. As our QTL mapping results suggested a complex polygenic inheritance for SBS-related traits, we also evaluated the prediction accuracy of two genomic prediction methods (GBLUP and BayesB). In general, we found that both explained considerably higher proportions of the genetic variance than the values obtained in QTL mapping with cross-validation. Nevertheless, the identified QTL regions could be used as a starting point for fine mapping and gene cloning.

Abbreviations

SBS	Stalk bending strength
RPR	Rind penetrometer resistance
NIRS	Near-infrared reflectance spectroscopy
FIAG	The fourth internode above ground
$F_{\max}$	The maximum load exerted to breaking

H. Hu and W. Liu contributed equally to this work.

Communicated by N. de Leon.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-013-2132-7) contains supplementary material, which is available to authorized users.

H. Hu · W. Liu · S. Chen (✉)
National Maize Improvement Center of China, China Agricultural University (West Campus), 2# Yuanmingyuan West Road, Beijing 100193, China
e-mail: chen368@126.com

H. Hu · L. Homann · F. Technow · A. E. Melchinger (✉)
Institute of Plant Breeding, Seed Science, and Population Genetics, University of Hohenheim, 70599 Stuttgart, Germany
e-mail: melchinger@uni-hohenheim.de

W. Liu · S. Chen
Beijing Key Laboratory of Crop Genetic Improvement, College of Agronomy and Biotechnology, China Agricultural University (West Campus), 2# Yuanmingyuan West Road, Beijing 100193, China

Z. Fu · C. Song · S. Li
Applicational Mechanics Department, College of Science, China Agricultural University (East Campus), 17# Qinghua East Road, Beijing 100083, China

H. Wang
Institute of Crop Science, Chinese Academy of Agricultural Sciences, 12# Zhongguancun South Street, Beijing 100081, China

$M_{\max}$	Breaking moment
$\sigma_{\max}$	Critical stress
Ld	Larger diameter of cross section
Sd	Smaller diameter of cross section
InL	Internode length
FreW	Fresh weight of the internode
DryW	Dry weight of the internode
InW	Internode water content
FreW/V	Fresh weight of internode per unit volume
DryW/V	Dry weight of internode per unit volume
ADL/V	Acid detergent lignin content per unit volume
CEL/V	Cellulose content per unit volume

Introduction

Maize plays an important role in securing the world's food supply, in animal husbandry and in the deep-processing industry. According to the statistics of the Food and Agriculture Organization (FAO), global production of maize in 2010 and 2011 amounted to 849 and 878 million tons, respectively, exceeding that of wheat and rice. However, stalk lodging causes yield losses in maize cultivation ranging between 5 and 20 % annually worldwide (Flint-Garcia et al. 2003).

The most direct way to evaluate stalk lodging resistance is counting the number of lodged plants at harvest. Since lodging strongly depends on the environmental conditions (Thompson 1963; Hu et al. 2012), it cannot always be reliably determined in field trials. Many studies (Zuber et al. 1961; Colbert et al. 1984; Jia et al. 1992; Gou et al. 2007) have found that stalk mechanical strength is positively correlated with stalk lodging resistance in the field. Enhancing overall mechanical strength in maize will make stalks stronger and ultimately reduce yield and grain quality losses (Ching et al. 2010). Since stalk lodging is extremely affected by the environmental conditions and, therefore, has often a low heritability, stalk mechanical strength can be viewed as a reliable indicator for evaluating stalk lodging resistance.

Several methods were developed and applied to evaluate stalk mechanical strength. Zuber and Grogan (1961) measured stalk crushing strength, whereas Sibale et al. (1992) used rind penetrometer resistance (RPR). Compared to these two traits, stalk bending strength (SBS) is more closely associated with stalk lodging in the field because under natural conditions, stalk lodging occurs when the stalk bending level exceeds the critical bending point (Yuan et al. 2002). Thus, bending deformation can be used to characterize the process of stalk lodging and the maximum load exerted to breaking ($F_{\max}$), the breaking moment ($M_{\max}$) and the critical stress ($\sigma_{\max}$) are three important parameters for characterizing bending strength (Gere and Timoshenko 1984).

Previous studies on SBS in different crops mainly focused on phenotypic correlations with stalk mechanical strength and the expression of candidate genes. In barley, Kokubo et al. (1989, 1991) used $\sigma_{\max}$ to determine the culm strength and studied the relationship between $\sigma_{\max}$ and culm components. In rice, Sun (1987) suggested the ratio of $F_{\max}$ to the product of culm length and ear weight as a measure to select for lodging resistance and Li et al. (2003) studied the relationship between $F_{\max}$ and the content of cellulose and lignin. In wheat, Ma (2009) found elevated expression levels of mRNA and proteins of the wheat COMT gene (*TaCM)* in cultivars with higher bending strength. In maize, Appenzeller et al. (2004) studied the relationship between $F_{\max}$ and the content of dry matter and cellulose per unit length in the stalk.

Mapping of quantitative trait loci (QTL) represents a powerful and well-established tool to analyze the genetic architecture of complex traits such as stalk mechanical strength. Flint-Garcia et al. (2003) and Hu et al. (2012) reported QTL for RPR in maize. For SBS in maize, only one QTL mapping study (Ching et al. 2010) analyzed QTL for $F_{\max}$ without cross-validation and did not consider $M_{\max}$ and $\sigma_{\max}$, which are also crucial parameters to characterize SBS according to the theory of biomechanics. Thus, the main goal of this study was to investigate the most relevant parameters ($F_{\max}$, $M_{\max}$, $\sigma_{\max}$) for SBS as well as three related morphological traits and seven stalk component traits in a population of 216 recombinant inbred lines (RILs) derived from a cross of two elite maize inbred lines evaluated in four environments. Our objectives were to (1) estimate genetic variances and heritability for $F_{\max}$, $M_{\max}$, $\sigma_{\max}$ and correlations of SBS with other traits; (2) detect and locate QTL for SBS and related traits; (3) evaluate the reliability of these QTL by means of cross-validation; and (4) compare the prospects of marker-assisted and genomic selection for SBS-related traits.

Materials and methods

Plant materials and field experiments

Stalks of high-oil maize genotypes have lower content of neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL), but higher in vitro dry matter digestibility (IVDMD) than normal maize genotypes (Bai et al. 2005; Wang 2009), which is one of the potential reasons for their higher lodging (Hu et al. 2012; Li et al. 2003; Zuber et al. 1977). Higher lodging of high-oil maize than normal maize was also found in practical high-oil maize breeding by ourselves and other breeders

(Lauer 1995). Therefore, high-oil maize was chosen as a parent susceptible to stalk lodging of our mapping population. Ce03005 with white kernels, a purple stem and an oil content of 8.52 % is a typical high-oil inbred line and suitable to serve as a lodging susceptible parent based on our previous research (Wang 2009). In contrast, the other parent line B73 is a widely used elite line from the stiff stalk heterotic group known for its excellent stalk lodging resistance. Therefore, our mapping population of 216 RILs was derived from the cross between B73 and Ce03005. The production and background of this population were described in detail in a companion study (Hu et al. 2012). In 2009 and 2010, the 216 RILs, the two parent lines and the F_1 generation were planted in the Changzhi, Shanxi Province, and Quzhou experiment station of China Agricultural University, Hebei Province, China. At each location, a randomized complete block design was utilized with two replications for each genotype. In each block, plants were sown in single rows, 3 m long, with a density of 60,000 plants/ha. Standard agronomic practices such as irrigation, fertilization and weeding were followed during the whole vegetation period to ensure a uniform stand.

Trait evaluation

Morphological trait measurements

Based on observations over many years of breeding practice in North China, we found that the fourth internode above ground (FIAG) of maize plants at the milky stage is highly sensitive to stalk lodging in the field. Hence, we evaluated three plants per plot at the milky stage with similar plant height and stem diameter. We measured the phenotypes for the FIAG with the same materials as in our companion study on RPR (Hu et al. 2012). For FIAG testing of each plant, the internode length (InL), the larger diameter of cross section (Ld) and the smaller diameter of cross section (Sd) were measured with an electronic micrometer. Afterward, the FIAG of the plants was cut with a garden shear on the same day for all lines. Fresh weight of the internode (FreW) was determined with an electronic scale.

Three-point bending test

The test pieces were placed on an electronic universal testing machine (RGT-2; Shenzhen, China). $F_{\max}$ is the maximum load that can be exerted to reach the critical breaking limit or the bending limit force. It was measured with a precision of 0.2 N and a displacement velocity of 20 mm/min as described by Kokubo et al. (1989). $M_{\max}$ represents the limit moment a stem can support before it breaks. $\sigma_{\max}$ is the maximal stress when the force moment (M) reaches M_{max} (Gere and Timoshenko 1984).

Near-infrared reflectance spectroscopy (NIRS) analysis

After completing the three-point bending test, the fresh internodes were immediately enzyme deactivated in a forced-air oven at 105 °C for 30 min, and then all samples were air dried at a bleachery for 10–14 days to measure dry weight (DryW). Simultaneously, internode water content (InW), fresh weight per unit volume (FreW/V) and dry weight per unit volume (DryW/V) were determined. Afterward, the three samples of FIAG collected in each plot were mixed, crushed, homogenized and then stored in a paper bag. After drying in a forced-air oven at 45 °C for 48 h, all samples were scanned using a near-infrared spectroscopy (NIRS) instrument (Vector 22/N, Bruker, Germany). Acid detergent lignin (ADL) and cellulose (CEL) contents were determined by NIRS using calibration equations developed for maize plants. Modified partial least squares (Shenk et al. 1991) were employed with the OPUS 6.0 Bruker software (Bruker) for setting up the calibration equations. Coefficients of determination for ADL and CEL for cross-validation (R^2_{cv}) and external validation (R^2_{val}) were described by Hu et al. (2012).

Phenotypic data analysis

Pairwise comparisons of means of the parents ($\bar{P}$), the F_1 generation and the RILs ($\overline{\mathrm{RIL}}$) were tested for significance with multiple t tests implemented in SAS PROC GLM (SAS Institute 2008). Analyses of variance were performed using the following linear mixed model:

$$Y_{ijk} = \mu + G_i + E_j + GE_{ij} + R_k(E) + \varepsilon_{ijk}.$$

Here, Y_{ijk} is the phenotypic value of genotype i in environment j and replication k; μ is the overall mean; G_i is the effect of inbred line i; E_j is the effect of environment j, treating each year–location combination as an environment; GE_{ij} is the interaction between inbred line i and environment j; R_k (E) is the block effect nested within each environment; and ε_{ijk} is the random error. Variance components of the genotypic variance (σ^2_{G}), genotype-by-environment interaction variance (σ^2_{GE}) and error variance (σ^2) were estimated by the restricted maximum likelihood (REML) method using PROC MIXED in SAS (SAS Institute 2008) and treating all terms in the model equation as random effects except μ. Heritability (h^2) on an entry-mean basis was estimated as described by Hallauer et al. (2010). Coefficients of phenotypic and genotypic correlation were estimated following Mode and Robinson (1959) using the software PlabStat (Utz 2010). For obtaining the phenotypic data for the QTL analysis, we calculated best linear unbiased estimates (BLUEs) across environments for all genotypes by treating the effects of lines as fixed. In contrast to best linear unbiased predictions (BLUPs), BLUEs are not shrunken toward the mean, and thus, avoid artifacts arising from twofold shrinkage (Piepho et al. 2012).

Molecular data collection and linkage map construction

Genomic DNA extraction and marker analyses were performed as described in detail in our companion study (Hu et al. 2012). Briefly, 129 polymorphic SSR markers were chosen for uniform coverage of the maize genome and employed for screening the RIL population to develop a genetic linkage map constructed using Icimapping 3.0 (Wang et al. 2010). An LOD threshold of 3.0 was used to assign markers to the same linkage group. Observed frequencies at each marker were tested against the expected Mendelian segregation ratio of 1:1 using a χ^2 test for goodness of fit and a Bonferroni correction for multiple tests. The number of heterozygous marker loci in each RIL was calculated by a simple program developed by R software (R Core Team 2012).

QTL analysis and cross-validation

Since the segregating population was an advanced generation RIL population, an additive genetic model was chosen for QTL analysis, using the BLUEs across environments as phenotypic data. We employed composite interval mapping (CIM) by the regression approach (Haley and Knott 1992) in combination with the use of cofactors (Jansen and Stam 1994; Zeng 1994). A two-step procedure was utilized for QTL detection. In the first step, cofactors were selected by stepwise multiple linear regression based on the Schwarz Bayesian Criterion (Schwarz 1978). Cofactor selection was performed using Proc GLMSELECT implemented in the statistical software SAS (SAS Institute 2008). In the second step, we calculated a P value for the F test with a full model (including marker effects) versus a reduced model (without marker effects). Then, the LOD score was calculated from the formula $\mathrm{LOD} = \frac{n}{2}\log_{10}\left[F\left(\frac{\mathrm{df}}{n-\mathrm{df}-1}\right)+1\right]$ (Broman and Sen 2009). An LOD threshold of 3.0 was used to determine QTL for all traits corresponding to an experiment-wise type I error of $P < 0.10$ based on 2,000 permutations (Doerge and Churchill 1996). Genome-wide scans for QTL were conducted using the statistical software R (R Core Team 2012). Because there were not suitable public software packages in R available for our case, we developed the procedure by ourselves. LOD support intervals were calculated by finding the location at either side of the estimated QTL location that corresponded to a decrease in 1 LOD score. The total proportion (p_G) of σ_G^2 explained by the detected QTL was calculated by fitting all QTL simultaneously in a linear model to obtain the R_{adj}^2 values. The ratio $p_G = R_{\mathrm{adj}}^2/h^2$ yielded the proportion of genotypic variance explained by the detected QTL (Utz et al. 2000).

Fivefold cross-validation was used to examine the reliability of QTL mapping results with 1,000 runs. In each run, 80 % of the lines were sampled and used as an estimation set (ES) for QTL detection; the remaining 20 % of the lines served as test set (TS) for validation of the detected QTL and estimation of the proportion ($p_{G,TS}$) of σ_G^2 explained as described in detail by Würschum et al. (2012).

Comparison of the prospects of phenotypic, marker-assisted and genomic selection

Under the assumption of the same selection intensity as well as equal cycle length for each selection method, the efficiency of marker-assisted selection based on the detected QTL relative to that of phenotypic selection is given by the ratio $p_{G,TS}$: h^2 (Lande and Thompson 1990). For evaluating the prospects of genomic selection, we compared the Bayesian GBLUP (Kärkkäinen and Sillanpää 2012) and BayesB (Meuwissen et al. 2001) methods in the implementations described by Technow et al. (2013) and Technow and Melchinger (2013), respectively. To allow for a direct comparison with h^2 and $p_{G,TS}$, we determined the proportion of the genetic variance ($p_{G,GS}$) in cross-validation averaged over 50 runs. Individual values of $p_{G,GS}$ were calculated as the square of the prediction accuracy, which in turn was computed as the Pearson correlation between predicted and observed values divided by the square root of the heritability. All computations for genomic prediction were conducted within the R statistical environment (R Core Team 2012).

Results

Phenotypic data analysis

No significant ($P < 0.05$) differences were found between the means of the two parents ($\bar{P}$) and the RIL population ($\overline{\mathrm{RIL}}$) for $F_{\max}$, $M_{\max}$ and $\sigma_{\max}$ as well as ten related stalk traits (Table 1). The means of the F_1 generation exceeded $\bar{P}$ and $\overline{\mathrm{RIL}}$ for $M_{\max}$, Ld, Sd, FreW, DryW and ADL/V. Further, the mean of the F_1 generation surpassed the two parents for $M_{\max}$ (Supplement Fig. 1). Little transgressive

Table 1 Means of parents ($\bar{P}$), F_1 generation (F_1) and RILs ($\overline{\text{RIL}}$), estimates of genotypic (σ^2_{G}), genotype × environment interaction (σ^2_{GE}) and error variances (σ^2_{ε}), heritability (h^2) and proportion of explained genetic variance ($p_{\text{G.GS}}$) in cross-validation for genomic selection methods GBLUP and BayesB of stalk bending-related traits

Trait	Unit	$\bar{P}$	F_1	$\overline{\text{RIL}}$	σ^2_{G}	σ^2_{GE}	σ^2_{ε}	h^2	$p_{\text{G.GS}}$[b]	
									GBLUP	BayesB
SBS traits										
$F_{\max}$	N	178.4a[a]	219.5a	181.1a	1,644.6**	999.9**	3,307.9	0.81	28.1	29.8
$M_{\max}$	N·m	4.86b	7.50a	4.93b	1.06**	0.744**	2.26	0.79	27.2	24.4
$\sigma_{\max}$	Mpa	10.0a	10.5a	10.3a	2.19**	1.60**	7.76	0.75	14.6	11.4
Geometric traits										
Ld	mm	17.6b	20.6a	18.0b	2.22**	1.03**	3.58	0.85	20.1	22.5
Sd	mm	1.59b	1.89a	1.59b	1.47**	0.716**	2.36	0.84	16.6	17.2
InL	cm	13.1ab	14.7a	11.3b	1.77**	1.26**	2.79	0.80	30.3	27.0
Stalk components traits										
FreW	g	36.8b	56.5a	33.4b	37.6**	31.0**	61.0	0.79	16.6	14.8
DryW	g	7.14b	9.06a	6.26b	1.36**	1.37**	2.47	0.75	16.8	13.5
InW	%	80.2a	72.75a	80.7a	3.98**	3.08**	6.58	0.79	35.7	38.0
FreW/V	g/cm^3*100	137.5a	132.8a	132.1a	6.01**	135.7**	591.4	0.51	9.7	11.7
DryW/V	g/cm^3*100	26.0a	22.7a	25.1a	6.33**	10.2**	28.5	0.63	27.2	22.8
ADL/V	g/dm^3	7.40b	9.61a	7.45b	1.32**	0.420**	2.96	0.73	27.4	25.1
CEL/V	g/dm^3	59.6ab	91.5a	58.4b	37.5**	21.6**	98.3	0.68	32.5	33.7

** Significant at $P < 0.01$

[a] Means in a line followed by the same letter are not significantly different from each other based on a *t* test at the 5 % probability level

[b] Means of $p_{\text{G.GS}}$ estimates over 50 runs of five-fold cross-validation

Table 2 Phenotypic (r_p) and genotypic (r_g) correlation coefficients between stalk strength traits and other traits

Trait	$F_{\max}$		$M_{\max}$		$\sigma_{\max}$	
	r_p	r_g	r_p	r_g	r_p	r_g
$F_{\max}$	–	–	0.86**	0.86^{++}	0.13	0.07
$M_{\max}$	0.86**	0.86^{++}	–	–	0.19**	0.13^{+}
$\sigma_{\max}$	0.13	0.07	0.19**	0.13^{+}	–	–
RPR	0.37**	0.37^{++}	0.46**	0.45^{++}	0.41**	0.46^{++}
Ld	0.67**	0.69^{++}	0.73**	0.75^{++}	−0.49**	−0.53^{++}
Sd	0.67**	0.69^{++}	0.77**	0.80^{++}	−0.42**	−0.45^{++}
InL	−0.37**	−0.41^{++}	0.14*	0.10^{+}	0.09	0.10^{+}
FreW	0.50**	0.52^{++}	0.81**	0.83^{++}	−0.14*	−0.17^{+}
DryW	0.30**	0.28^{++}	0.67**	0.65^{++}	0.03	−0.01
InW	0.28**	0.37^{++}	0.18**	0.27^{++}	−0.29**	−0.29^{++}
FreW/V	0.40**	0.45^{++}	0.20**	0.23^{++}	0.41**	0.45^{++}
DryW/V	0.01	−0.09	−0.03	−0.12^{+}	0.53**	0.56^{++}
ADL/V	−0.06	−0.12^{+}	−0.08	−0.14^{+}	0.38**	0.37^{++}
CEL/V	0.01	−0.08	−0.10	−0.21^{++}	0.31**	0.24^{++}

*, ** Phenotypic correlation coefficient significant at the 0.05 and 0.01 probability level, respectively

$^{+}$, $^{++}$ Genotypic correlation coefficient exceeds once or twice its standard error, respectively

segregation was observed for $F_{\max}$ and $M_{\max}$; however, a relatively large proportion of lines fell outside the range of the parental lines for $\sigma_{\max}$ (47.4 %). Estimates of σ^2_{G} and σ^2_{GE} were highly significant for all traits. The heritabilities ranged between 0.51 for FreW/V and 0.85 for Ld, with moderately high values for $F_{\max}$ (0.81), $M_{\max}$ (0.79) and $\sigma_{\max}$ (0.75).

Correlation among traits

$F_{\max}$ was positively correlated with $M_{\max}$, Ld, Sd, FreW, DryW, InW and FreW/V, but negatively with InL (Table 2). $M_{\max}$ showed similar correlations with all traits with the exception of a positive correlation with InL. By contrast, $\sigma_{\max}$ was negatively correlated with Ld, Sd, FreW

Table 3 Position, estimated effect of QTL, proportion of the genotypic variance (p_G) explained by QTL, QTL frequency (Freq) and proportion of genotypic variance($p_{G,TS}$) explained by the detected QTL in the test set by fivefold of cross-validation with 1,000 runs for SBS and related traits

Trait	Bin	Position[a] (cM)	Flanking markers	LOD	α[b]	p_G %	Cross-validation	
							Freq (%)	$p_{G,TS}$ (%)
F_{max}								
	5.02	65.6 (58.9, 72.4)	umc2115, umc1447	4.8	−16.3	22.4	55.8	14.2
	10.04	56.1 (51.3, 59.7)	umc1697, phi084	5.0	13.9		85.2	
M_{max}								
	5.03	83.0 (72.4, 93.7)	umc1447, umc1171	4.5	−0.42	26.1	68.8	7.2
	8.03	70.9 (63.3, 78.6)	phi100175, umc1562	3.7	0.37		44.8	
	10.03	55.0 (48.1, 62.9)	phi050, umc1697	3.1	0.29		23.0	
σ_{max}								
	5.03	93.2 (82.5, 105.8)	umc1447, umc1171	4.0	0.63	17.2	61.6	3.7
	10.06	114.4 (106.6, 124.3)	umc1993, bnlg1450	3.0	0.50		23.8	
Ld								
	5.03	87.1 (81.5, 93.7)	umc1447, umc1171	11.6	−0.11	25.5	100.0	24.8
Sd								
	5.03	88.1 (82.5, 94.7)	umc1447, umc1171	9.9	−0.08	22.1	100.0	22.0
InL								
	1.03	69.5 (63.0, 76.1)	umc1403, umc1169	3.6	0.52	18.1	43.4	2.3
	6.07	250.4 (241.6, 259.1)	phi299852, phi123	4.0	−0.54		35.8	
FreW								
	5.03	90.1 (77.5, 102.8)	umc1447, umc1171	3.5	−2.5	18.1	52.6	5.5
	8.03	67.9 (62.3, 75.5)	phi100175, umc1562	4.0	2.2		52.2	
DryW								
	8.03	67.9 (62.3, 74.5)	phi100175, umc1562	4.5	0.49	11.6	64.4	6.4
InW								
	6.01	116.8 (115.2, 117.5)	bnlg1867, umc2056	8.4	−0.74	24.5	95.0	18.8
	7.04	174.2 (170.6, 175.2)	dupssr13, phi116	6.7	−0.71		34.4	
DryW/V								
	6.01	116.8 (114.1, 118.9)	bnlg1867, umc2056	4.6	0.01	14.1	77.0	7.7
ADL/V								
	5.03	99.2 (89.6, 108.9)	umc1447, umc1171	5.5	0.054	21.3	82.2	7.9
	10.02	28.2 (20.3, 35.2)	umc2034, umc2016	3.9	−0.042		40.2	
CEL/V								
	3.05	93.9 (86.4, 102.4)	phi053, umc1539	4.0	0.26	21.1	50.0	10.6
	5.03	101.3 (88.6, 106.8)	umc1447, umc1171	3.7	0.22		43.8	

[a] QTL support interval given in brackets

[b] Estimate of allele effect

and InW, and positively with FreW/V, DW/V, ADL/V and CEL/V. RPR measured for the internode below the primary ear (IBPE) was positively correlated with F_{max}, M_{max} and σ_{max} ($P < 0.05$).

Marker and QTL analyses

Only a small number (14) of markers showed significant segregation distortion. These distorted markers were distributed on seven chromosomes (Supplement Fig. 2). The observed heterozygosity at the 129 markers was <5 % in 89 % of the lines and more than 45 % of the lines were heterozygous at <1 % of the loci (Supplement Fig. 3).

The number of QTL detected for F_{max}, M_{max} and σ_{max} was two, three and two, explaining 22.4, 26.1 and 17.2 % of σ^2_G, respectively (Table 3). The QTL for F_{max} and M_{max} had overlapping support intervals on chromosome 10. Likewise, the QTL for M_{max} and σ_{max} had common flanking markers in bin 5.03 adjacent to the QTL region of $F_{\max}$ on chromosome 5. One major QTL was also detected for both Ld and Sd in bin 5.03, which explained 25.5 and 22.1 % of σ_G^2, respectively. The two QTL for InL had no common support intervals with those for Ld and Sd, and accounted together for 18.1 % of σ_G^2. Except for FreW/V, where no QTL was found, one or two QTL were detected for the other stalk component traits, explaining between 11.6 and 24.5 % of σ_G^2.

Cross-validation

For most QTL identified in the full data set, high frequencies of re-detection were observed in the 1,000 runs of cross-validation (Table 3). The frequency of QTL re-detection was 100 % for the QTL detected for Ld and Sd, and ranged between 55.8 and 68.8 % for the QTL detected for $F_{\max}$, $M_{\max}$ and $\sigma_{\max}$ on chromosome 5. The $p_{G,TS}$ values estimated from the TS ranged from 2.3 % for InL to 24.8 % for Ld (Table 3). For the three SBS traits, $F_{\max}$ showed slightly higher $p_{G,TS}$ values (14.2 %) compared to $M_{\max}$ and $\sigma_{\max}$.

In genomic selection, estimates of $p_{G,GS}$ were for both GBLUP and BayesB (Table 1) considerably higher than those of $p_{G,TS}$ (Table 3). The only exceptions were Ld and Sd, where the mapped QTL explained in cross-validation a slightly greater proportion of σ_G^2. The differences in $p_{G,GS}$ between GBLUP and BayesB were usually small, with BayesB yielding better results for six of the traits and GBLUP for the remaining seven traits.

Discussion

RILs performance and heritability of stalk bending strength traits

The contrast between $\bar{P}$ and $\overline{\mathrm{RIL}}$ provides an estimate of the net contribution of additive × additive epistatic interactions across loci (Melchinger et al. 2007). Since this difference was not significant for any trait, we conclude that QTL underlying SBS-related traits display mainly additive gene action or positive and negative additive × additive epistatic effects canceled each other in the sum. The contrast $F_1 - \bar{P}$ indicated significant mid-parent heterosis for 6 out of the 13 traits, and $M_{\max}$ even showed strong high-parent heterosis, suggesting dominance in addition to additive gene action for these traits. The large proportion of transgressive segregation for $\sigma_{\max}$ is mainly attributable to the small phenotypic difference between the two parents that were not selected for $\sigma_{\max}$ and shows that both parents harbor positive and negative genes for this trait. A similar phenomenon was reported for RPR in a segregating population of B73 × M47 (Flint-Garcia et al. 2003).

According to the theory of mechanics of materials (Gere and Timoshenko 1984), SBS is determined by $F_{\max}$, $M_{\max}$ and $\sigma_{\max}$. In rice, Sun (1987) reported $h^2 = 0.84$ for $F_{\max}$, which is in close agreement with the estimates of $F_{\max}$, $M_{\max}$ and $\sigma_{\max}$ in our study. For RPR, h^2 estimates ranged between 0.81 and 0.92 in five different maize populations (Flint-Garcia et al. 2003; Hu et al. 2012), suggesting that stalk strength-related traits have generally a high heritability. Heritability is an important factor in determining the power of QTL detection (Charcosset and Gallais 1996). In this study, the heritability exceeded 0.73 for ten traits and only one trait (FreW/V) had a medium h^2 of 0.51. Thus, all traits were measured with high precision and this provided a solid basis for QTL mapping.

Correlation and co-locations of QTL among traits

Sun (1987) found that $F_{\max}$ positively correlated with stem diameter, DryW and dry weight per unit length in rice. Appenzeller et al. (2004) concluded that structural dry matter explained approximately 80 % of $F_{\max}$ in maize. In our experiment, we observed positive correlations of $F_{\max}$ with Ld, Sd and DryW, which are consistent with the findings of both studies and the theory of material mechanics in that $F_{\max}$ has a tight relation to the geometric attributes of materials (Gere and Timoshenko 1984). Positive correlations were also observed between $F_{\max}$ and FreW as well as between InW and FreW/V, which implies that water content is crucial for the stalk strength of maize plants at the milky stage, because the turgor pressure from water in cells translates into increased bending strength of the stalk. These findings are supported by other studies on stalk lodging in maize (Stojsin et al. 1991; Gao et al. 2003). In addition, one QTL of $F_{\max}$ shared one flanking marker with QTL for Ld, Sd and FreW, which suggests either pleiotropy or close linkage of QTL as an explanation of these correlations.

$M_{\max}$ showed similar correlations as $F_{\max}$ with the other traits and both traits were also tightly correlated with each other. Therefore, we recommend measuring only $F_{\max}$ in practical breeding programs for improving stalk lodging resistance, because calculation of $M_{\max}$ is based on $F_{\max}$ and the InL. $\sigma_{\max}$ was negatively correlated with Ld, Sd and InW, but positively correlated with DryW/V, ADL/V and CEL/V. Similar results between $\sigma_{\max}$ and stalk chemical components were also reported by Kokubo et al. (1989) in barley. These correlations can be explained by the theory of material mechanics in that $\sigma_{\max}$ is hardly influenced by the geometric properties of the stalk, but reveals the essential attributes of the material that can be reflected by the dry matter content and stalk components per unit volume in our case (Gere and Timoshenko 1984). This hypothesis is corroborated by the overlapping QTL support intervals found between $\sigma_{\max}$ and ADL/V, CEL/V. While the positive correlation of SBS traits with ADL/V and CEL/V has no negative effect in the production of grain maize (Hansey and Leon 2011), it is expected to have negative consequences on the digestibility of stover in the production of forage maize, but this warrants further research.

In a companion study with the same plant material, Hu et al. (2012) detected nine QTL for RPR, covering nine chromosomes. A comparison with the QTL results for $F_{\max}$, $M_{\max}$ and $\sigma_{\max}$ in this study revealed overlapping QTL support intervals in adjacent bins 10.03 and 10.04 for RPR, $F_{\max}$ and $M_{\max}$, and in bin 8.03 for RPR and $M_{\max}$. This suggests that RPR and SBS are influenced by some common genes. In addition, RPR correlated positively with $F_{\max}$ and $M_{\max}$, which provides further indication for a common genetic basis of these traits. In addition, RPR primarily reflects the rind mechanical strength, which contributes mainly to the mechanical strength of the intact internode (Zuber et al. 1980).

We observed segregation distortion between adjacent markers in regions on chromosomes 1, 3, 8 and 9 (Supplemental Fig. 2). It is well understood that segregation distorted loci will affect estimates of recombination frequencies between marker loci. Since chromosome regions with segregation distortion can also harbor QTL, deleting these markers from the linkage map can result in missing QTL. Xu (2008) concluded that if segregation distortion is present but ignored, this will slightly decrease the power of QTL detection. Moreover, QTL mapping is hardly affected if the distorted markers are not closely linked with any QTL (Zhang et al. 2010). Since no close linkage was found between any QTL and distorted markers in our study, we conclude that distorted markers had little influence on the QTL detection power in our study.

Chromosome regions harboring QTL for stalk bending strength traits

An investigation of Ching et al. (2010) on SBS in maize with 189 non-Stiff Stalk (NSS) lines reported results on $F_{\max}$ for which QTL were detected on chromosomes 1, 5 and 9; the QTL located in bins 5.02 and 5.03 was the most important one and overlaps with the QTL for $F_{\max}$ on chromosome 5 found in our study. Since this QTL was also detected with high frequencies in the 1,000 cross-validation runs, this corroborates with our conclusion that bins 5.02 and 5.03 harbor candidate genes for $F_{\max}$. A further QTL for $F_{\max}$ in bin 10.04, found with a high frequency in cross-validation, was not detected by Ching et al. (2010).

No reports were available on QTL for $M_{\max}$ and $\sigma_{\max}$. For both traits, we detected QTL on the same chromosomes as the two QTL of $F_{\max}$, but on different positions. The QTL for $M_{\max}$ and $\sigma_{\max}$ on chromosome 10 had relatively low detection frequencies in cross-validation, illustrating the necessity for further research with a larger population size and a higher marker density. However, the QTL in bins 5.02 and 5.03 had high detection frequencies in cross-validation for $F_{\max}$, $M_{\max}$ and $\sigma_{\max}$, suggesting that this region merits further investigations for finding candidate genes underlying SBS.

The main application of QTL mapping results is marker-assisted selection. For improving SBS, phenotypic evaluations of $F_{\max}$, $M_{\max}$ and $\sigma_{\max}$ are expensive and labor intensive. Therefore, molecular marker-based technologies offer a more efficient way for improving SBS. In this study, the ratio of $p_{\mathrm{G,TS}}$: h^2 for the three traits was <0.60, indicating that conventional phenotypic selection is more efficient than the QTL-based marker-assisted selection for improving SBS traits. Similar results were reported for corn borer resistance traits (Bohn et al. 2001; Papst et al. 2004; Ordas et al. 2010). Nevertheless, the chromosome regions in bins 5.02 and 5.03 harboring QTL for $F_{\max}$, $M_{\max}$ and $\sigma_{\max}$ could be used as a starting point for fine mapping and gene cloning for SBS-related traits. Since the detected QTL explained only a moderate proportion of σ_{G}^2 in cross-validation, we conclude that SBS-related traits show a complex polygenic inheritance. This hypothesis is supported by the considerably greater values of $p_{\mathrm{G,GS}}$ in comparison to $p_{\mathrm{G,TS}}$, when using genomic prediction methods such as GBLUP or BayesB. Thus, genomic selection might be the method of choice to improve these traits because it allows taking QTL with small effects into account that otherwise remain undetected in QTL mapping (Lorenz et al. 2011).

Conclusions

We found generally a high heritability and little evidence of epistasis for the SBS-related traits in the B73 × Ce03005 derived population of RILs. $F_{\max}$ and $M_{\max}$ were positively correlated with Ld, Sd, InW, DryW and FreW of intact internode, whereas $\sigma_{\max}$ was negatively correlated with Ld, Sd and water content, but positively correlated with DryW/V, ADL/V and CEL/V. These correlations among traits could be partly explained by co-locations of corresponding QTL and were consistent with the theory of material mechanics. We detected two chromosome regions harboring QTL for SBS: one in bin 5.02 and 5.03 for $F_{\max}$, $M_{\max}$ and $\sigma_{\max}$, and another one in bin 10.04 for $F_{\max}$. All these QTL had high re-detection frequencies in cross-validation. Marker-assisted selection based on the QTL mapping results was comparatively less efficient than conventional phenotypic selection and genomic selection, but further studies on fine mapping and gene cloning for stalk bending strength traits seem promising based on our findings. Altogether, we conclude that the genetic architecture of SBS-related traits is highly polygenic. Since these traits are also difficult and labor intensive to measure in a breeding program, it seems that genomic selection is the most promising avenue to improve these traits, given the tremendous progress and decreasing prices for new sequencing technologies and SNP assays that provide the required genome coverage for this approach (Jannink et al. 2010).

Acknowledgments We thank Lianying Yu, Baohua Liu and Yan Luo of China Agricultural University for their help in the three-point bending test. We also thank H. Fritz Utz, Tobias Schrag and Xuefei Mi for their suggestions on the data analysis. This research was supported by grants from the Modern Maize Industry Technology System Foundation of China (No. nycytx-02) to S. Chen, DFG, Grant No. 1070/1, International Research Training Group "Sustainable Resource Use in North China" to A.E. Melchinger and National Natural Science Foundation of China (No. 10972234) to Z. Fu.

References

Appenzeller L, Doblin M, Barreiro R, Wang H, Niu X, Kollipara K, Carrigan L, Tomes D, Chapman M, Dhugga KS (2004) Cellulose synthesis in maize: isolation and expression analysis of the cellulose synthase (*CesA*) gene family. Cellulose 11:287–299

Bai Q (2005) Inheritance of stover quality traits and their determination by near-infrared reflectance spectroscopy (NIRS) in silage maize. Dissertation, China Agricultural University

Bohn M, Groh S, Khariallah MM, Hoisington DA, Utz HF, Melchinger AE (2001) Re-evaluation of the prospects of marker-assisted selection for improving insect resistance to *Diatraea* spp. in tropical maize by cross validation and independent validation. Theor Appl Genet 103:1059–1067

Broman KW, Sen S (2009) A Guide to QTL Mapping with R/qtl. Springer

Charcosset A, Gallais A (1996) Estimation of the contribution of quantitative trait loci (QTL) to the variance of a quantitative trait by means of genetic markers. Theor Appl Genet 93:1193–1201

Ching A, Rafalski JA, Luck S, Butruilie MG (2010) Genetic loci associated with mechanical stalk strength in maize. United States Patent Publication. No. US2010/0015623A1

Colbert TR, Darrah LL, Zuber MS (1984) Effect of recurrent selection for stalk crushing strength of agronomic characteristics and soluble stalk solids in maize. Crop Sci 24:473–478

Doerge RW, Churchill GA (1996) Permutation tests for multiple loci affecting a quantitative character. Genetics 142:285–294

Flint-Garcia SA, Jampatong C, Darrah LL, Mcmullen MD (2003) Quantitative trait locus analysis of stalk strength in four maize populations. Crop Sci 43:13–22

Gao M, Guo K, Yang Z, Li X (2003) Study on mechanical properties of corn stalk. Trans CSAM 34:47–49

Gere JM, Timoshenko SP (1984) Mechanics of Materials. Van Nostrand Reinhold Company Ltd, New York

Gou L, Huang J, Zhang B, Li T, Sun Y, Zhao M (2007) Effect of population density on stalk lodging resistant mechanism and agronomic characteristics of maize. Acta Agronomica Sinia 33:1688–1695

Haley CS, Knott SA (1992) A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. Heredity 69:315–324

Hallauer AR, Carena MJ, Miranda Filho JB (2010) Quantitative Genetics in Maize Breeding. Springer, New York

Hansey CN, Leon N (2011) Biomass yield and cell wall composition of corn with alternative morphologies planted at variable densities. Crop Sci 51:1005–1015

Hu H, Meng Y, Wang H, Liu H, Chen S (2012) Identifying quantitative trait loci and determining closely related stalk traits for rind penetrometer resistance in a high-oil maize population. Theor Appl Genet 124:1439–1447

Jannink J, Lorenz AJ, Iwata H (2010) Genomic selection in plant breeding: from theory to practice. Brief Funct Genomics 9:166–177

Jansen RC, Stam P (1994) High resolution of quantitative traits into multiple loci via interval mapping. Genetics 136:1447–1455

Jia Z, Bai Y (1992) Study on identification of lodging in maize inbred line. China seeds 3:30–32

Kärkkäinen H, Sillanpää M (2012) Back to basics for Bayesian model building in genomic selection. Genetics 191:969–987

Kokubo A, Kuraishi S, Sakurai N (1989) Culm strength of barley correlation among maximum bending stress, cell wall dimensions, and cellulose content. Plant Physiol 91:876–882

Kokubo A, Sakurai N, Kuraishi S, Takeda K (1991) Culm brittleness of barley (*Hordeum vulgare* L.) mutants is caused by smaller number of cellulose molecules in cell wall. Plant Physiol 97:509–514

Lande R, Thompson R (1990) Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics 124:743–756

Lauer J (1995) High-Oil Corn: advantages and risks. Field Crops 28.31–3

Li Y, Qian Q, Zhou Y, Yan M, Sun L, Zhang M, Fu Z, Wang Y, Han B, Pang X, Chen M, Li J (2003) BRITTLE CULM1, which encodes a COBRA-like protein, affects the mechanical properties of rice plants. Plant Cell 15:2020–2031

Lorenz AJ, Chao S, Asoro FG, Heffner EL, Hayashi T, Iwata H, Smith HI, Sorrells ME, Jannink JL (2011) Genomic selection in plant breeding: knowledge and prospects. Adv Agron 110:77–123

Ma Q (2009) The expression of caffeic acid 3-O-methyltransferase in two wheat genotypes differing in lodging resistance. J Exp Botany 60:2763–2771

Melchinger AE, Utz HF, Piepho HP, Zeng Z, Schön CC (2007) The role of epistasis in the manifestation of heterosis: a system-oriented approach. Genetics 177:1815–1825

Meuwissen TH, Hayes BJ, Goddard M (2001) Prediction of total genetic value using genome-wide dense marker maps. Genetics 157:1819–1829

Mode CJ, Robinson HF (1959) Pleiotropism and the genetic variance and covariance. Biometrics 15:518–537

Ordas B, Malvar R, Santiago R, Butron A (2010) QTL mapping for Mediterranean corn borer resistance in European flint germplasm using recombinant inbred lines. BMC Genomics 11:174–183

Papst C, Bohn M, Utz HF, Melchinger AE, Klein D, Eder J (2004) QTL mapping for European corn borer resistance (Ostrinia nubilalis Hb.), agronomic and forage quality traits of testcross progenies in early-maturing European maize (*Zea mays* L.) germplasm. Theor Appl Genet 108:1545–1554

Piepho H-P, Mohring J, Schulz-Streek T, Ogutu JO (2012) A stage-wise approach of the analysis of multi-environment trials. Biometrical J 00:1–17

SAS Institute Inc (2008) SAS user's guide, version9.2. SAS institute Cary, NC

Schwarz G (1978) Estimating the dimension of a model. Ann Stat 6:461–464

Shenk JS, Westerhaus MO (1991) Population structuring of near infrared spectra and modified partial least square regression. Crop Sci 31:1548–1555

Sibale EM, Darrah LL, Zuber MS (1992) Comparison of two rind penetrometers for measurement of stalk strength in maize. Maydica 37:111–114

Stojsin R, Ivanovic M, Kojic L, Stojsin D (1991) Inheritance of grain yield and several stalk characteristics significant in resistance to stalk lodging maize (*Zea mays* L.). Maydica 36:75–81

Sun X (1987) Studies on the resistance of the culms of rice to lodging. Scientia Agricultura Scinica 20:32–37

R Core Team (2012) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, http://www.R-project.org/

Technow F, Melchinger AE (2013) Genomic prediction of dichotomous traits with Bayesian logistic models. Theor Appl Genet 126:1133–1143

Technow F, Bürger A, Melchinger AE (2013) Genomic prediction of northern corn leaf blight resistance in maize with combined or separated training sets for heterotic groups. G3 3:197–203

Thompson DL (1963) Stalk strength of corn as measured by crushing strength and rind thickness. Crop Sci 3:323–329

Utz HF (2010) PLABSTAT: a computer program for statistical analysis of plant breeding experiments. Institute of Plant Breeding, Seed Science, and Population Genetics, University of Hohenheim, Stuttgart, Germany

Utz HF, Melchinger AE, Schön CC (2000) Bias and sampling error of the estimated proportion of genotypic variance explained by quantitative trait loci determined from experimental data in maize using cross validation and validation with independent samples. Genetics 154:1839–1849

Wang H (2009) QTL analysis and genetic relationship between kernel compositions and stalk nutrition quality traits in high oil maize population. Dissertation, China Agricultural University

Wang J, Li H, Zhang L, Li C, Meng L (2010) Users' Manual of QTL IciMapping V3.0, Beijing

Würschum T, Liu W, Gowda M, Maurer HP, Fischer S, Schechert, Reif JC (2012) Comparison of biometrical models for joint linkage association mapping. Heredity 108:332–340

Xu S (2008) Quantitative trait locus mapping can benefit from segregation distortion. Genetics 180:2201–2208

Yuan Z, Feng B, Zhao A, Liang A (2002) Dynamic analysis and comprehensive evaluation of crop-stem lodging resistance. Trans CSAE 18(6):30–31

Zeng Z (1994) Precision mapping of quantitative trait loci. Genetics 136:1457–1468

Zhang L, Wang S, Li H, Deng Q, Zheng A, Li S, Li P, Li Z, Wang J (2010) Effects of missing marker and segregation distortion on QTL mapping in F_2 populations. Theor Appl Genet 121:1071–1082

Zuber MS, Grogan CO (1961) A new technique for measuring stalk strength in corn. Crop Sci 1:378–380

Zuber MS, Colbert TR, Bauman LF (1977) Effect of brown-midrib-3 mutant in maize (*Zea mays*) on stalk strength. Z Pflanzenzuecht 79:310–314

Zuber MS, Colbert TR, Darrah LL (1980) Effect of recurrent selection for crushing strength on several stalk components in maize. Crop Sci 20:711–717