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ORIGINAL PAPER

Identifying quantitative trait loci and determining closely related stalk traits for rind penetrometer resistance in a high-oil maize population

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Abstract Stalk lodging in maize causes annual yield losses between 5 and 20% worldwide. Many studies have indicated that maize stalk strength significantly negatively correlates with lodging observed in the field. Rind penetrometer resistance (RPR) measurements can be used to effectively evaluate maize stalk strength, but little is known about the genetic basis of this parameter. The objective of this study was to explore a genetic model and detect quantitative trait loci (QTL) of RPR and determine relationships between RPR and other stalk traits, especially cell wall chemical components. RPR is quantitative trait in nature, and both additive and non-additive effects may be important to consider for the improvement of RPR. Nine additive-effect QTLs covering nine chromosomes, except chromosome 5, and one pair of epistatic QTLs were detected for RPR. *CeSA11* involved in cellulose synthesis and *colorless2* involved in lignin synthesis were identified as possible candidate genes for RPR. Internode diameter (InD), fresh weight of internode (FreW), dry weight of internode (DryW), fresh weight and dry weight as well as cell wall components per unit volume significantly positively correlated with RPR. The internode water content (InW) significantly negatively correlated with RPR. Notably, these traits significantly correlated with RPR, and the QTLs of these traits co-localized with those of RPR. The corresponding results obtained from correlation analysis and QTL mapping suggested the presence of pleitropism or linkage between genes and indicated that these different approaches may be used for cross authentication of relationships between different traits.

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Abbreviations

ADF/V	Acid detergent fiber content per unit volume of internode
ADL/V	Acid detergent lignin content per unit volume of internode
CEL/V	Cellulose content per unit volume of internode
CF/V	Crude fiber content per unit volume of internode
DryW	Dry weight of internode
DryW/V	Dry weight of internode per unit volume
FreW	Fresh weight of internode
FreW/V	Fresh weight of internode per unit volume
IBPE	The internode below the primary ear
InD	Internode diameter
InL	Internode length
InW	Internode water content
NDF/V	Neutral detergent fiber content per unit volume of internode

QTL	Quantitative trait loci
RIL	Recombinant inbred line
RPR	Rind penetrometer resistance
SCS	Stalk crushing strength

Introduction

Maize has become an important crop not only for food and fodder in animal husbandry and the deep processing industry, but also for industrial and energy uses worldwide as the application of bio-energy using maize stalk as raw material increases. In China, the planting areas of maize have exceeded that of rice, and maize has become the most widely planted crop (Tong 2010; Weng 2010). However, stalk lodging causes maize yield losses estimated in the range of 5–20% annually worldwide (Flint-Garcia et al. 2003). In China, stalk lodging in maize has become the major factor restricting maize yield and planting density (Gou et al. 2010).

The most commonly used method to evaluate stalk lodging resistance is to count lodged plants at harvest. Although this method is effective in discriminating plants resistant to lodging from those susceptible to lodging, it is seriously dependent on the environmental conditions (Thompson 1963). Because it is possible that, materials become upgraded, optimum environmental conditions do not occur or not at satisfactory frequencies to test the breeding effects. Therefore, the development of crop evaluation techniques independent of environmental factors is necessary. Zuber and Grogan (1961) developed a stalk crushing strength (SCS) method to evaluate stalk lodging and found that SCS significantly positively correlates with lodging resistance in the field. McRostie and MacLachlan (1942) used the manual penetrometer to evaluate stalk strength and stalk lodging. Meanwhile, in wheat, Ma (2009) used a three-point bending method to evaluate the second basal internode strength and found that the plant material resistant to stalk lodging had higher stalk strength than the susceptible one. Jia and Bai (1992) also developed a field bending method, which could be used in evaluation of stalk bending strength and stalk lodging. The rind penetrometer resistance (RPR) method is to use a modified electronic rind penetrometer with a probe to penetrate the rind of maize stalk, and the maximum value during the process of penetrating will be shown on the screen of the penetrometer (Sibale et al. 1992). Compared to other methods mentioned above, the RPR method is simple, rapid and most importantly does not damage the stalk (Hu et al. 2000). In addition, many studies have indicated that RPR significantly negatively correlates with actual stalk lodging in the field (McRostie and MacLachlan 1942; Gou et al. 2007). In this study, we focused on RPR as an indicator of maize stalk strength.

Previous studies on RPR in maize mainly focused on phenotypic analysis and the relationship between RPR and morphological traits. McRostie and MacLachlan (1942) found that RPR decreases from low to high internode, and RPR significantly positively correlates with stalk lodging in the field. Sibale et al. (1992) estimated the coefficient of variation (CV) of RPR to be 10.5% in two synthetic maize populations. The phenotypic correlation between RPR and morphological traits has also been reported (Gou et al. 2010; Yao 2003; Li et al. 2004).

Compared to phenotypic analysis, less research has been performed on the genetic basis of RPR. Feng et al. (2009) fitted RPR to a model utilizing a pair of additive-dominance major genes plus an additive-dominance-epitasis polygene (D Model) with a six-generation maize population. Yao (2003) estimated that the general combining ability (GCA) and specific combining ability (SCA) could explain 48.7 and 51.3% of the genetic variance, respectively. Flint-Garcia et al. (2003) detected eight, ten, eight and nine single-effect quantitative trait loci (QTLs) and four, two, zero, and five epistatic interactions for RPR in four maize populations.

However, there are currently no reported investigations on the relationship between RPR and cell wall chemical components of rind, and little is known about the genetic basis, QTLs and potential candidate genes for RPR. Therefore, our objectives in this study were to: (1) explore the genetic model for RPR; (2) identify the QTLs associated with RPR and related stalk traits; (3) determine the stalk traits, especially cell wall components of rind, significantly correlated with RPR. We expected the results of this work to provide basis for stalk strength improvement through: (1) selection of maize breeding strategy; (2) molecular marker-assisted selection; (3) selection of morphological traits and cell wall components.

Materials and methods

Plant materials

Based on many years of experience on high-oil maize breeding and observations in the field, we found that high-oil maize is susceptible to lodging and a high-oil inbred line might be an ideal material for studies on lodging resistance and stalk strength. We obtained such a high-oil inbred line Ce03005 (kernel oil content 8.52%), susceptible to lodging, by self-crossing from ethylmethane-sulphonate-treated pollen of a normal hybrid ND108 (kernel oil content 4.34%, Chen and Song 2002). The 216 lines used in this study comprised the F_8, F_9 and F_{10} generations of the

RIL population derived from B73 × Ce03005. B73 is a widely used normal line (kernel oil content 4.34%). The creation of the experimental population commenced in 2001, as described by Han et al. (2008) and Wang et al. (2010a). Briefly, the procedure was as follows: an F_2 population was derived from a cross between the high-oil maize line Ce03005 and normal line B73. F_2 plants were self-pollinated to generate F_3 ear kernels. Subsequently, F_3 seeds were planted in ear lines with three replications, and three self-pollinated plants of each line were bulked to generate F_4 kernels until F_{10}.

Field experiments

In 2008, 2009 and 2010, the 216 RILs, their parental lines and F_1 were planted in the Changzhi, Shanxi Province and Quzhou experiment station of China Agricultural University (CAU), Hebei province, China. At each location, a randomized complete block design with two replications of each generation was used. For each replication, plants were sown in single-row plots 3 m long, at a density of 60,000 plants/ha. Unified management measures, such as irrigation, fertilization and weed cutting were applied during the whole growth period.

Evaluation of traits

Based on previous studies (Yao 2003; Ma 2009) and several years of survey and statistical data of lodging rate in the field, we propose that milk stage is suitable for stalk strength evaluation. In addition, our study results also indicated that both the stalk strength and some of stalk components were obviously lower in milky stage than those in the flowering stage in different materials (unpublished data). In this stage, we chose 3–5 plants with similar plant height and stem diameter in every line, which were marked with labels at the internode below the primary ear (IBPE) for testing. For each labeled plant, RPR was measured in the middle of the flat side of the IBPE with the electronic penetrometer (YN-JD, Xunjie companay, Zhengzhou), and RPR was determined by the mean of five tests. Internode length (InL) and diameter (InD) were measured with electronic micrometers. For each sample of internode, larger diameter of cross section was recorded as InD. When evaluations of the traits were completed, the IBPE samples were cut with garden shears, and the fresh weight measured with electronic scales. Subsequently, the fresh internodes were immediately enzyme-deactivated in a forced-air oven at 105°C for 30 min and then air-dried at bleachery for 10–14 days. Each sample was measured for dry weight and then the rind was separated from each sample. The rind of the samples in the same lines were crushed together, homogenized and stored in a paper bag. All samples were scanned using a near-infrared reflectance spectroscopy (NIRS; VECTOR 22/N, Bruker, Germany) after drying in a forced-air oven at 45°C for 48 h. Neutral detergent fiber (NDF), crude fiber (CF), acid detergent fiber (ADF), cellulose (CEL), and acid detergent lignin (ADL) content were estimated by NIRS. NIRS prediction equations were developed for maize plants using modified partial least squares (Shenk and Westerhaus 1991) using OPUS 6.0 (Bruker, Germany) software. The coefficients of determination for cross-validation (R^2_{cv}) and external validation (R^2_{val}) were, respectively, 0.95 and 0.92 for NDF, 0.93 and 0.92 for ADF, 0.89 and 0.89 for CF, 0.95 and 0.95 for FIB, and 0.94 and 0.93 for ADL (Bai et al. 2004, 2006; Wu 2004; Wang 2009).

Phenotypic data analysis

The data sets from 3 years and two locations were viewed as six independent macro environments. Phenotypic data analysis was based on mean values of traits in lines across six independent macro environments. *t* tests and descriptive statistical analyses were determined using software SPSS 17.0 software. Variance analysis, heritability estimation and calculation of coefficient of phenotypic and genetic correlation were estimated using PlabStat 3A (Utz 2010).

Variance analysis was based on the following liner model:

$$\text{Model E} + \text{R} : G + G + EG + \varepsilon$$

E is environmental effect, G is genetic effect, EG is gene × environment interaction, R symbolizes the replication effect, ε is random effect, and R is nestled within G. E, R and G are treated as random effect in this model.Broad-sense heritability (h^2_B) was estimated by the following formulas:

$$h^2_B = \sigma^2_G/\sigma^2_P$$
$$\sigma^2_P = \sigma^2_G + \sigma^2_{GE}/e + \sigma^2_\varepsilon/re,$$

σ^2_G = variance component of genotype, σ^2_P = variance of phenotype, σ^2_{GE} = variance component of genotype × environment interaction, σ^2_ε = variance component of random error, e = number of environment, and r = number of replicates.

Genotypic data collection

Young leaves from F_8 plants were harvested, freeze-dried with liquid N_2, ground to powder and stored at −20°C in individually labeled vials. Genomic DNA extraction was performed using a CTAB method as described by Hoisington et al. (1994).

SSR analysis was conducted as reported by Senior and Heun (1993) using publicly available primers from the MaizeGDB (http://www.maizegdb.org). The RIL population DNA was amplified using co-dominant segregation SSR markers, and a genetic linkage map was constructed using Icimapping 3.0 (Wang et al. 2010b).

QTL analysis

QTLNetwork 2.2 (Yang et al. 2010) was used for QTL analysis of mean values of traits in lines across environments. QTLNetwork 2.2 is a software program for mapping QTL with epistatic effects and QTL-by-environment (QE) interaction effects in DH, RIL, BC_1, BC_2, F_2, IF_2 and B_xF_y populations, and for graphical presentation of QTL mapping results. The software was developed based on the mixed-model-based composite interval mapping (MCIM) method (Wang et al. 1999; Zhu 2000).

Results

Phenotypic data analysis

In addition to RPR, five typical stalk phenotypic traits were evaluated in this study (Table 1). The Ce03005 parental line had higher RPR, InL and DryW than those of the B73 parental line; however, B73 had higher InD, FreW and InW. For all these traits, the differences between B73 and Ce03005 were significant at the 0.01 probability level by a two-tailed *t* test (Table 1).

For all traits, means of the recombinant inbred line (RIL) population were distributed between those of the parental inbred lines B73 and Ce03005. All the traits had moderate or high h_B^2, which ranged from 0.69 to 0.91 (Table 1). Particularly, among all the traits, RPR had the highest heritability (0.91). These results indicated all the traits had good genetic basis, and were suitable for QTL analysis.

QTL analysis

To construct a genetic linkage map, 502 simple sequence repeats (SSR) markers from the whole maize genome were assessed with the two parental lines, from which 143 were polymorphic markers and 129 were used to develop the genetic map. The markers covered a total of 2016.52 centimorgan (cM) in length, with an average distance of 15.6 cM between markers (Fig. 1).

For RPR, one dimension scanning for additive effect QTL and partial two dimensional scanning for epistatic QTL were conducted genome-wide using QTLNetwork 2.2 with a step length of 1 cM. Nine additive effect QTLs covering nine chromosomes, except chromosome 5, and a pair of epistatic QTLs were detected (Tables 2, and 3). In general, for each of the QTLs for RPR, the percentage of contribution to the phenotypic variation was below 15%. For additive effect QTLs, the QTL at bin 3.06 had the largest contribution to phenotypic variance (12.43%), and the QTL at bin 4.08 had both additive and epistatic effects (Table 2). For six of the nine additive effect QTLs, the parental line Ce03005 donated beneficial alleles for increasing RPR, which was consistent with the phenotype of Ce03005 having a higher RPR than that of B73.

For other stalk traits, the additive effect QTLs sharing common flanking markers or had overlapping confidence intervals with those of RPR focused on InD, FreW and DryW (Table 2). The QTLs for InD at bin 4.08, InW at bin 7.04, InD, FreW and DryW at bin 8.03 shared flanking markers in common with those of RPR, and the QTLs for InD, FreW and DryW at bin 10.04 had overlapping confidence intervals with that of RPR. Among all these QTLs, the QTL for InD at bin 4.08 had the largest percentage (14.72%) of contribution to phenotypic variation. Five pairs of epistatic QTLs were detected, of which at least one QTL for each pair shared common flanking markers with additive effect or epistatic effect QTLs for RPR (Table 3). Two of the five pairs of epistatic QTLs were related to InD.

Table 1 Characteristics of B73, Ce03005, and the RIL population derived from B73 × Ce03005

Trait	Unit	B73 (mean ± SD)	Ce03005 (mean ± SD)	*t* test (*t* value)	RIL			Variance		
					Mean ± SD	Min	Max	σ_g^2	σ_{ge}^2	h_B^2
RPR	N/dm^2	12.98 ± 1.13	15.39 ± 2.91	−3.29**	14.04 ± 4.90	2.35	30.06	2.63	1.01	0.91
InL	cm	13.68 ± 1.52	17.70 ± 3.13	−4.60**	14.26 ± 2.56	4.90	28.36	1.71	0.78	0.83
InD	cm	1.56 ± 0.13	1.24 ± 0.16	7.27**	1.49 ± 0.22	0.70	2.39	0.02	0.01	0.88
FreW	g	25.91 ± 4.34	21.58 ± 6.43	3.76**	24.89 ± 8.26	6.23	57.76	22.91	13.16	0.73
DryW	g	5.49 ± 1.16	6.30 ± 1.68	−2.67**	5.52 ± 1.73	1.31	13.47	1.05	0.68	0.71
InW	%	78.52 ± 2.83	70.42 ± 2.99	8.80**	77.36 ± 3.59	49.05	88.68	3.51	2.11	0.69

SD standard deviation, h_B^2 broad-sense heritability, *Min* minimum, *Max* maximum, σ_g^2 variance component of genotype, σ_{ge}^2 variance component of genotype × environment interaction

* $P < 0.05$; ** $P < 0.01$

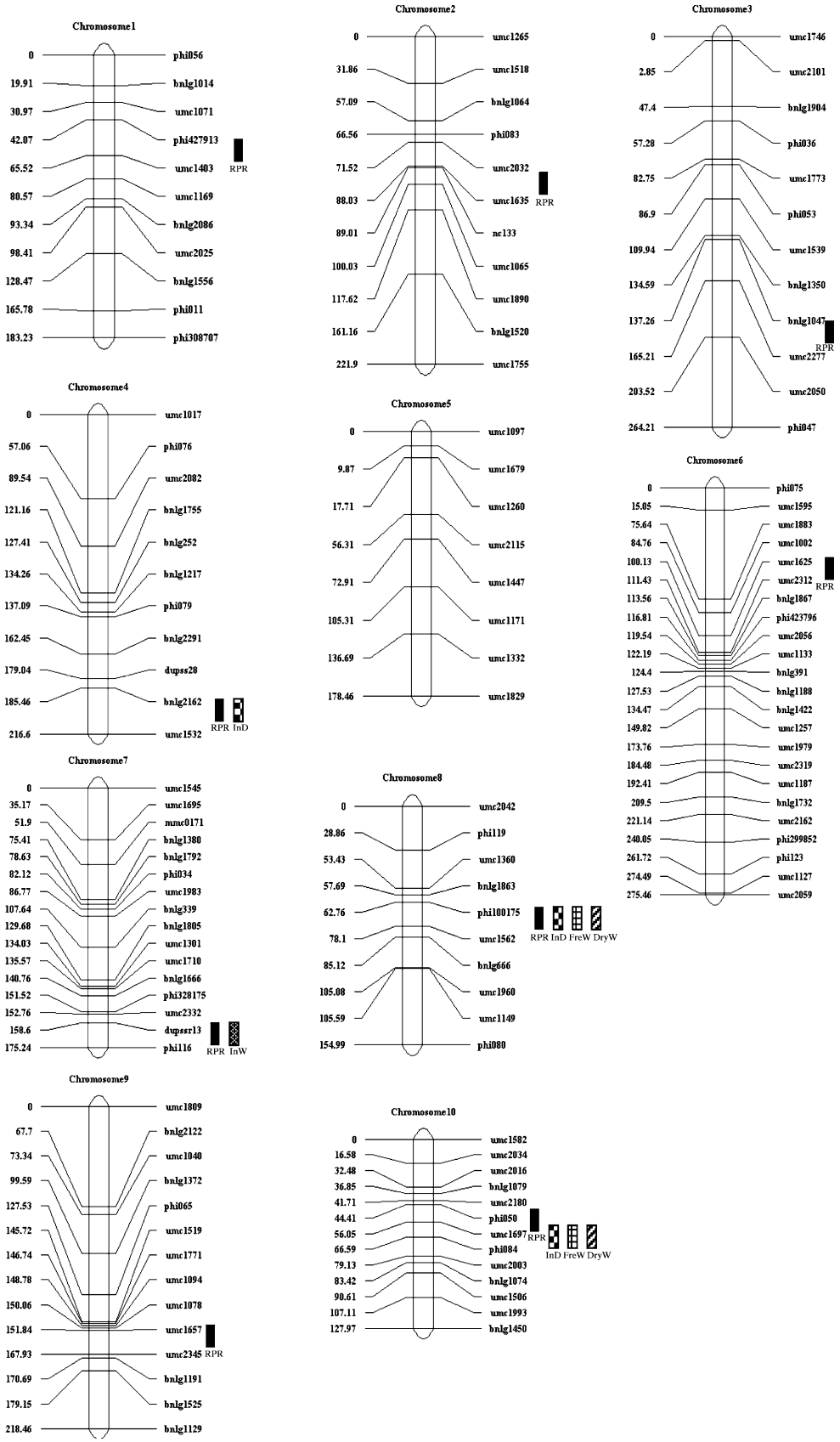

Fig. 1 Linkage map and relative position of QTLs in RIL population derived from B73 × Ce03005

Table 2 Position, additive effect and heritability of QTLs for RPR and QTLs for stalk and kernel traits sharing common flanking markers with those of RPR

QTLCode[a]	Bin[b]	Flanking markers	Position[c]	Range[d]	A[e]	*P* value[f]	q^2(a) (%)[g]
qRPR1	**1.01**	**phi427913-umc1403**	**50.1**	**42.1–56.1**	**63.02**	**0.00**	**7.72**
qRPR2	**2.04**	**umc2032-umc1635**	**87.5**	**84.5–93.0**	**−27.82**	**0.00**	**1.50**
qRPR3	**3.06**	**bnlg1047-umc2277**	**150.3**	**143.3–155.3**	**−79.98**	**0.00**	**12.43**
qRPR4	**4.08**	**bnlg2162-umc1532**	**200.5**	**192.5–208.5**	**59.22**	**0.00**	**6.82**
qInD4	4.08	bnlg2162-umc1532	200.5	195.5–206.5	0.07	0.00	14.72
qRPR6	**6.01**	**umc1625-umc2312**	**107.1**	**101.1–112.4**	**−24.27**	**0.00**	**1.15**
qRPR7	**7.04**	**dupssr13-phi116**	**166.6**	**159.6–172.6**	**−42.41**	**0.00**	**3.50**
qInW7	7.04	dupssr13-phi116	174.6	169.6–174.6	0.47	0.00	2.66
qRPR8	**8.03**	**phi100175-umc1562**	**74.8**	**66.8–80.1**	**−35.75**	**0.00**	**2.48**
qInD8	8.03	phi100175-umc1562	73.8	66.8–84.1	−0.03	0.00	2.09
qFreW8	8.03	phi100175-umc1562	69.8	63.8–75.8	−1.91	0.00	7.38
qDryW8-1	8.03	phi100175-umc1562	67.8	61.7–74.8	−0.26	0.00	2.99
qRPR9	**9.05**	**umc1657-umc2345**	**163.8**	**157.8–169.9**	**35.39**	**0.00**	**2.43**
qRPR10	**10.03**	**phi050-umc1697**	**52.4**	**48.4–63.0**	**−38.25**	**0.00**	**2.84**
qInD10	10.04	umc1697-phi084	57.0	50.4–62.0	−0.03	0.00	3.20
qFreW10	10.04	umc1697-phi084	58.0	49.4–63.0	−1.20	0.00	2.90
qDryW10	10.04	umc1697-phi084	61.0	46.4–66.6	−0.24	0.00	2.47

Bold values indicate the parameters of QTLs for RPR

[a] Code for QTL associated with traits and the chromosome number

[b] Bins are estimated based on the proximity of the QTL to the left flanking marker and the bin location of markers in the MaizeGDB

[c] Genetic map position by cM

[d] Confidence interval of the QTL estimated by QTLNetwork2.2

[e] Additive effects estimated by QTLNetwork2.2

[f] Probability of *t* test between the value of additive effect and zero

[g] Heritability of the QTL

Table 3 Position, additive effect and heritability of epistatic QTLs for RPR and epistatic QTLs for stalk and kernel traits sharing the common flanking markers with those of RPR

Trait	Chr_i[a]	Flanking markers	Position_i[b]	Chr_j[c]	Flanking markers	Position_j[d]	AA[e]	*P* value[f]	q^2(aa)[g]
RPR	**4**	**bnlg2162-umc1532**	**200.5**	**8**	**umc2042-phi119**	**9.0**	**−63.68**	**0.00**	**0.08**
InD	**2**	**umc2032-umc1635**	**71.5**	9	umc1809-bnlg2122	23.0	−0.10	0.00	0.30
DryW	**3**	**bnlg1047-umc2277**	**137.3**	10	umc1697-phi084	61.0	−0.20	0.00	0.02
InL	**3**	**bnlg1047-umc2277**	**144.3**	5	umc1260-umc2115	18.7	0.45	0.00	0.05
InW	**3**	**bnlg1047-umc2277**	**156.3**	9	umc1809-bnlg2122	38.0	−1.90	0.00	0.44
InD	1	phi011-phi308707	176.8	**8**	**phi100175-umc1562**	**73.8**	0.03	0.00	0.03

The parameters in bold type are those of QTLs sharing common flanking markers with epistatic QTLs of RPR

[a] Chromosome of the first QTL of the additive–additive effect (AA) interaction

[b] Genetic map position of the first QTL of the A–A interaction by cM estimated by QTLNetwork2.2

[c] Chromosome of the second QTL of the A–A interaction

[d] Genetic map position of the second QTL of the A–A interaction by cM estimated by QTLNetwork2.2

[e] A–A interaction effects estimated by QTLNetwork2.2

[f] Probability of *t* test between the value of A–A effect interaction effect and zero

[g] Heritability of the A–A interaction effect

Notably the other three pairs of epistatic QTLs shared common flanking markers with the QTL of RPR at bin 3.06, which had the largest additive effect among all the QTLs for RPR.

Phenotypic and genetic correlation analysis

InL, InD, FreW, DryW and InW were primarily evaluated for correlation with RPR. Through the phenotypic and

genetic correlation analysis, we found that InD, FreW and DryW significantly positively correlated with RPR, while InW significantly negatively correlated with RPR (Table 4). This result indicated that the plant with higher dry matter content and lower water content may have higher RPR, which was consistent with the observation that between the two parental inbred lines, Ce03005 with a lower water content and higher DryW, had higher RPR than that of B73 (Table 1).

The relationship between RPR and cell wall components of rind, which were the main components of dry matter were also investigated (Table 4). We found that RPR significantly positively correlated with acid detergent fiber content (ADF/V), acid detergent lignin content (ADL/V), crude fiber content (CF/V); cellulose content (CEL/V), and neutral detergent fiber content (NDF/V) per unit volume of internode.

Discussion

No consistent heritability and genetic model of RPR has been obtained, since the calculations depend on experimental materials, experimental design and environmental conditions which may vary between studies (Kong 2007). Feng et al. (2009) calculated the genetic model of RPR of the third internode above the ground in maize with six populations of different generations, including F_1, F_2, BC_1, BC_2, P_1, and P_2. Their results indicated that RPR is quantitative trait in nature and fits the model utilizing a pair of additive-dominance major genes plus an additive-dominance-epistasis polygene. Yao (2003) calculated that the additive effects and non-additive effects explained 48.7 and 51.3% of genetic variance, respectively, based on a complete diallele crossing experiment. Using four $F_{2:3}$ maize populations, Flint-Garcia et al. (2003) estimated h_B^2 of RPR to range from 0.81 to 0.92 across environments and found that additive and dominance effects explained 37.27–65.24% of phenotypic variation. In this study, the estimation of h_B^2 was approximately 0.91 across six environments, consistent with the results of Flint-Garcia et al. (2003) that RPR has high h_B^2. The additive effect and non-additive effect could explain 41 and 59% of genetic variance, respectively, similar to the results of Yao (2003). All the findings mentioned above suggested that RPR is a quantitative trait with a good genetic basis, and both additive and non-additive effects may be important in the improvement of RPR. These results may also provide important reference information for breeders to select a suitable breeding strategy to improve stalk mechanical strength when using RPR as an indicator.

In the only study reporting analysis of QTL for RPR based on SSR markers as mentioned above, Flint-Garcia et al. (2003) detected several single-effect QTLs and epistatic QTLs in each of four $F_{2:3}$ maize populations. However, among all the QTLs, only one single-effect QTL detected in the population derived from M47 × B73 could explain the phenotypic variation above 15%, and only one region on chromosome 3 contained overlapping confidence intervals from all four populations. According to the positions of publicly available markers reported in the maize genetics and genomics database (MaizeGDB), this overlapping confidence interval was positioned approximately at the region between bin 3.04 and bin 3.08. In this study, we used a high-oil RIL population derived from B73 × Ce03005 to locate the QTLs for RPR. Nine additive effect QTLs covering nine chromosomes, except chromosome 5, and a pair of epistatic QTLs were detected genome-wide. For each QTL detected, the percentage of contribution to phenotypic variation was lower than 15%, which agreed with the results of Flint-Garcia et al. (2003). The consistent results from five different experimental populations indicated that a lack of major QTL for RPR and this trait may be controlled by multiple genes with small effects. Among all the QTLs detected in this study, the QTL at bin 3.06 (the left marker is at bin 3.06, the right marker is at bin 3.07) having the largest effect could explain 12.43% of the phenotypic variation, and the QTL at bin 4.08 had both additive and epistatic effects. It is worth noting that the QTL at bin 3.06 had an over-lapping confidence interval with the only common region from four populations detected by Flint-Garcia et al. (2003). Bernardo et al. (2010) located QTLs of Mediterranean corn borer resistance at bins 1.02, 3.05 and 8.05 in a RIL population of flint germplasm. Roussel et al. (2002) located

Table 4 Coefficient of phenotypic and genetic correlation between RPR and maize stalk traits

Trait name	Unit	R_p	R_g
InL	m	0.06	−0.06
InD	m	0.39**	0.38^{++}
FreW	g	0.44**	0.42^{++}
DryW	g	0.55**	0.55^{++}
InW	%	−0.24**	-0.26^{++}
FreW/V	g/cm^3	0.37**	0.52^{++}
DryW/V	g/cm^3	0.48**	0.83^{++}
ADF/V	g/cm^3	0.52**	0.90^{++}
ADL/V	g/cm^3	0.49**	0.72^{++}
CF/V	g/cm^3	0.49**	0.84^{++}
CEL/V	g/cm^3	0.51**	0.83^{++}
NDF/V	g/cm^3	0.50**	0.92^{++}

R_p coefficient of phenotypic correlation, R_g coefficient of genetic correlation

*/$^+$ $P < 0.05$; **/$^{++}$ $P < 0.01$

QTLs of ADL and in vitro cell wall digestibility (IVNDFD) at bins 3.05 and 3.06, respectively. Wang (2009) detected QTLs of lignin and cellulose content at bin 4.08. By comparing with the QTL mapping results from other related studies, bin 3.06, bin 4.08 and nearby regions may be important for RPR.

Therefore, we focused on bin 3.06, bin 4.08 and nearby regions to find potential candidate genes for RPR. We searched the region of bin 3.06 and nearby region in Maize GDB for potential candidate genes and found *CesA11*, which is a member of a gene family involved in cellulose synthesis. The *CesA11* locus was estimated to be between positions 201,187,932 and position 201,304,625 (116,693 base pairs in length) on chromosome 3-based on the map: IBM2 2008 Neighbors 3 (http://www.maizegdb.org/cgibin/displaylocusrecord.cgi?id=953721). Brien et al. (2011) found that *CesA11* along with *CesA10* and *CesA12* play important roles in the cell wall strength and are involved in secondary cell wall biosynthesis, although not exclusively. In another important candidate region, the *colorless 2* (*c2*) gene was founded at bin 4.08 in MaizeGDB. The locus *c2* was estimated to be between positions 191,044,122 and 193,347,979 (2,303,857 base pairs in length) on chromosome 4 based on the map: IBM2 2008 Neighbors 4 (http://www.maizegdb.org/cgi-bin/displaylocusrecord.cgi?id=12102). The genes *c2* and *whp1* have been shown to play an important role in the synthesis of lignin precursors (Flint-Garcia et al. 2003). The results of many researches (Zuber et al. 1977; Ma 2009) indicated that lignin plays an important role in stalk strength in different crops. Based on the results of different analyses mentioned above, the gene *CesA11* at bin 3.07 and the gene *c2* at bin 4.08 were identified as potential possible candidate genes for RPR.

Compared to studies on the genetics of RPR, there have been relatively more reports on the relationship between RPR and morphological traits. Yao (2003) found that RPR significantly positively correlated with dry weight of internode per unit volume (DryW/V) and rind thickness; however, no significant phenotypic correlations were found between RPR and InL or InD. Using 20 hybrids, Li et al. (2003) found a significant positive phenotypic correlation between RPR and FreW or InL, but none between RPR and InD. For flowering time, Gou et al. (2010) found a significant positive phenotypic correlation of RPR with FreW, DryW, and DryW/V and a negative correlation with InW in four hybrids differing in lodging resistance. Appenzeller et al. (2004) found that dry matter per unit length explained 80% of stalk strength. In this study, we found InD, FreW, DryW, FreW/V and DryW/V significantly positively correlated with RPR, while InW was negatively correlated with RPR, which was largely consistent with the combined results of other authors mentioned above (except for InD). However, there were apparent differences between all these studies, which we hypothesized to have resulted from different genetic materials, experimental designs and methods for statistical analysis. Despite the different results, the common view was that DryW/V significantly positively correlated with RPR.

No information had previously been reported on the relationships between RPR and cell wall components in maize rind. In rice, Li et al. (2003) found that compared to wild-type plants, the breaking force of *brittle culm1* mutants decreased to 43–52% with a reduction in cellulose content and an increase in lignin content. Wang et al. (2006) found that cellulose plays a more important role in SCS than lignin in wheat. However, Ma (2009) concluded that lignin determines the physical strength of wheat plants, as a lower lignin content causes stems to be soft. He also determined that wheat plants with higher bending resistance exhibit higher expressions of mRNA, protein as well as enzyme activity from *TaCM*, the full-length cDNA for caffeic acid 3-*O*-methyltransferase (COMT) involved in lignin synthesis. These obvious differences between all these studies indicate no agreement has been reached on effect of cellulose and lignin on stalk strength in rice and wheat. In maize, Appenzeller et al. (2004) found 85% of stalk bending strength could be explained when only cellulose per unit length was considered. Zuber et al. (1977) found a significant reduction of SCS in *bm3* mutants compared to normal plants, because of the low lignin content in *bm3* mutants. In this study, we found that the level of the five cell wall components content (measured per unit volume) mentioned above significantly positively correlated with RPR. Considering maize has a very different stalk structure from rice and wheat with hollow jointed stems, we inferred that the content of both cellulose and lignin may be especially important for stalk strength in maize and this was supported by the identification of genes related to cellulose and lignin synthesis as candidates for RPR. In addition, for many plant cells, cellulose usually accounts for approximately 20–30% of the dry weight of the primary walls and 49–90% of the secondary walls, depending on the cell type (Taylor et al. 1999). In some cells, lignin may be incorporated into the cell wall, enhancing its mechanical strength (Li et al. 2003).

Meanwhile, we also noticed that the QTLs for morphological traits also co-located to the QTLs of RPR to which they were significantly correlated. Bernardo et al. (2010) also found the QTLs of stalk tunnel length co-located with the QTLs of plant height, grain humidity and yield; however, the correlations between stalk tunnel length and grain humidity and between stalk tunnel length and yield were weak. In this study, strong phenotypic and genetic correlation corresponded to the co-location of QTLs for RPR and other stalk traits. We concluded that this observation was not merely a coincidence but rather

confirmation of the relationships between different traits from two different methods. The results also indicated there may be presence of pleiotropism or linkage between genes, because we noted that if several traits had co-located QTLs, of which the alleles donated by same parent, these traits always had significant positive correlation; the alleles donated by different parents, those traits always had significant negative correlation.

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