Genotypic Correlation and Multivariate QTL Analyses for Cell Wall Components and Resistance to Stalk Tunneling by the European Corn Borer in Maize

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ABSTRACT

Correlations between concentrations of cell wall components (CWCs) in the leaf sheath and stalk and resistance to stalk tunneling by the European corn borer (ECB) [*Ostrinia nubilalis* (Hübner)] have been reported in some maize (*Zea mays* L.) populations. Evaluations of resistance to ECB stalk tunneling (ECB-ST) and concentrations of neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) have been performed on recombinant inbred lines (RILs) developed from the cross of maize inbred lines B73 (susceptible to ECB-ST, low to moderate CWC concentrations) and DE811 (resistant to ECB-ST, high CWC concentrations). The objective of this study was to estimate genotypic correlations between ECB-ST and CWC concentrations and compare locations and effects of quantitative trait loci (QTL) for those traits. Genotypic correlations between ECB-ST and CWCs were not significant, but clustering of QTL for ECB-ST and CWCs was observed. Negative genotypic correlations between ECB-ST and CWC concentrations were observed at some loci, and resistance to ECB-ST may be associated with a subset of the QTL observed for CWCs and ADF in particular. Resistance to ECB-ST may also be associated with starch concentration in the stalk, which could explain the detection of resistance alleles contributed by B73. Examination of temporal differences in CWC and starch concentrations, and marker-assisted transfer of select alleles, could provide more information on mechanisms of resistance to ECB-ST.

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Abbreviations: ADF, acid detergent fiber; ADL, acid detergent lignin; CWCs, cell wall components; ECB, European corn borer; ECB-ST, ECB stalk tunneling; IVDMD, in vitro dry matter digestibility; NDF, neutral detergent fiber; QTL, quantitative trait loci; RILs, recombinant inbred lines; WFISIHI, Wisconsin fiber silica high; WFISILO, Wisconsin fiber silica low.

The biological basis of resistance to stalk tunneling by the European corn borer (ECB) [*Ostrinia nubilalis* (Hübner)], a major pest of temperate maize (*Zea mays* L.), is not well under-stood. The borer normally has two generations per year in the U.S. cornbelt, with the first generation feeding primarily on leaf tissue and the second generation feeding primarily on leaf sheath, shank, and stalk tissues (Mason et al., 1996). Resistance to leaf feeding by ECB in temperate maize is usually conferred by the chemical 2,4–dihidroxy–7–methoxy–1,4–benzoxazin–3–one (DIMBOA), but the chemical is not associated with resistance to ECB-ST, as concentrations of DIMBOA decrease as the plant matures (Klun et al., 1967, 1970; Klun and Robinson, 1969). One possible mechanism of resistance to leaf sheath and stalk feeding is increased concentrations of CWCs, particularly fiber and lignin, in the leaf sheath and stalk.

Elevated levels of fiber and lignin have been correlated with resistance to ECB-ST in several studies. The BS9(CB) population was developed for use in a recurrent selection program for resistance to both generations of ECB (Klenke et al., 1986). Highly significant linear correlated responses were observed for leaf sheath NDF, ADF, and ADL over four cycles of selection, indicating a possible genetic association between concentrations of CWCs and resistance to ECB-ST. In addition, negative genotypic correlations were observed between ECB-ST and concentrations of NDF, ADF, and ADL in F_3 lines of B73 × DE811, and heritability estimates for all cell wall components ($H^2 > 70\%$) in this population were within the range of the heritability for resistance to ECB-ST ($H^2 = 74\%$; Beeghly et al., 1997).

To clarify the association between CWC concentrations and ECB-ST, selection for CWC concentrations was performed in BS9(CB) Cycle 2 (BS9(CB) C2), Wisconsin fiber silica low (WFISILO), and Wisconsin fiber silica high (WFISIHI), to produce BS9(CB) C2-Lo and WFISILO C0, C1, and C2 (selected for lower concentrations of CWCs) and BS9(CB) C2-Hi and WFISIHI C0, C1, and C2 (selected for higher concentrations of CWCs) (Ostrander and Coors, 1997). The populations WFISILO and WFISILH differed significantly for CWC concentrations, but not for ECB-ST. Selection for decreased CWCs in BS9(CB) C2-Lo and WFISILO resulted in increased ECB stalk tunneling, but selection for higher concentrations of CWCs did not consistently result in lower ECB stalk tunneling. The BS9(CB) C2-Hi population differed significantly from BS9(CB) C2-Lo for stalk NDF, ADF and lignin concentrations, and ECB stalk tunneling, but selection in WFISIHI for increased CWC concentrations did not decrease ECB stalk tunneling. The results suggest that selection for low CWC concentrations may increase susceptibility to second-generation ECB, and, conversely, selection for ECB resistance may adversely affect the nutritional value of maize harvested as silage (Ostrander and Coors, 1997).

Genotypic correlations and comparisons of locations of QTL for ECB-ST and CWC concentrations have been reported for RILs of B52 × B73 (Cardinal and Lee, 2005). In that population, genotypic correlations between ECB-ST and CWC concentrations ranged from −0.27 to −0.44, and a majority of the QTL observed for ECB-ST were linked to QTL for CWCs, indicating that CWCs may play some role in expression of resistance to ECB-ST in the RILs of B52 × B73.

Quantitative trait loci for resistance to ECB-ST and CWC concentrations have been mapped in RILs of B73 × DE811 (Krakowsky et al., 2004, 2005, 2006). B73 is susceptible to ECB-ST and has low to intermediate concentrations of CWCs, while DE811 is resistant to ECB-ST and has high concentrations of CWCs.

The primary objective of this study was to estimate genotypic correlations between, and compare locations and effects of QTL for, ECB-ST and CWC concentrations to provide a better understanding of the relationship between these traits. Multivariate QTL mapping was used to estimate the amount of genotypic covariance between ECB-ST and CWC associated with specific genome regions. A secondary objective was to compare the genetic correlations and QTL estimates from this population to those reported by Cardinal and Lee (2005) for RILs of B73 × B52, which were evaluated for the same traits.

MATERIALS AND METHODS

The univariate QTL analyses of the B73 × DE811 population have been reported previously (Krakowsky et al., 2004, 2005, 2006). Briefly summarizing the materials and methods from those reports, 200 RILs of B73 × DE811, along with both parental inbreds, were planted at two locations in 1998 and one location in 1999. The ECB-ST and CWC concentrations were evaluated in all environments. Two stalk-tunneling traits were originally analyzed due to the correlation between ECB-ST and anthesis: ECB-ST and ECB-ST adjusted for maturity. Adjustment of ECB-ST for differences in maturity was performed by including anthesis as a covariate in the model for calculation of least-square means for ECB-ST (Krakowsky et al., 2004). The resulting adjusted ECB-ST least-square means were used for calculation of genotypic correlations and comparisons of QTL herein. In addition, NDF and NDF adjusted for ADF (NDF adjusted) were reported for both stalk and sheath CWCs (Krakowsky et al., 2005, 2006). To separate the effects of different CWCs, NDF adjusted for ADF was used for calculation of genotypic correlations and comparisons of QTL herein. The ADL was not included in the analyses herein due to the low genetic variances observed in both the sheath and stalk (Krakowsky et al., 2005, 2006). Genotypic covariances and correlations (r_g) and the standard errors of the correlations were calculated herein among traits by applying standard procedures (Mode and Robinson, 1959). The necessary computations were performed using the MANOVA statement in PROC GLM of the software package SAS, with entries and environments treated as random effects (SAS Institute, Inc., 1999).

To investigate the effects of QTL on ECB-ST adjusted for maturity and individual CWCs simultaneously, multivariate multiple regression analysis was conducted on two traits, using marker loci as regressor variables, as described by Robertson-Hoyt et al. (2006). The initial regression analysis model contained one marker locus nearest each QTL peak from the final QTL models for each trait. Multivariate analysis was conducted using the MANOVA option of Proc GLM in SAS version 8.2 (SAS Institute, Inc., 1999). A single degree of freedom contrast was defined for each marker locus or epistatic interaction. The initial model was overspecified and included several nonsignificant terms; therefore, backward selection was used to develop a model in which all terms had significant effects. The marker contrast with the largest Type III p value of Wilks' lambda statistic (which tests the null hypothesis of no marker effect on the two traits simultaneously) was dropped from the model. The resulting new model was tested in the same way, and this process continued iteratively until all marker loci had significant ($p = 0.05$) effects according to Wilks' lambda statistic.

The genotypic covariance between the two traits associated with each marker locus in the final model was estimated by the method of moments, using the Type III mean cross products for each marker contrast obtained from the Proc GLM analysis. To obtain unbiased estimates of the covariances, the coefficients of the covariance components were based on the equations of Charcosset and Gallais (1996). These estimates were used to calculate the proportion of total genotypic covariance between the two traits due to each marker locus.

RESULTS AND DISCUSSION

The genotypic correlations between ECB-ST adjusted for maturity and leaf sheath NDF adjusted and ADF (−0.08 [standard error (S.E.) = 0.10] and 0.02 [S.E. = 0.07], respectively) and between ECB-ST adjusted for maturity and stalk NDF adjusted and ADF (0.25 [S.E. = 0.26] and 0.13 [S.E. = 0.13], respectively) were not significantly different from zero and were lower than those reported by Beeghly et al. (1997) for leaf sheath in the F_3 lines of B73 × DE811. The genotypic correlations reported by Cardinal and Lee (2005) for ECB-ST with leaf sheath and stalk ADF in the RILs of B73 × B52 were −0.44 and −0.36, respectively, which are much higher than those observed herein. The NDF adjusted for ADF was not evaluated in the RILs of B73 × B52. These results suggest that the associations between ECB-ST adjusted for maturity and concentrations of CWCs observed in other studies may not be applicable to this population. In most of the cases this may be due to genetic heterogeneity, but that would not apply to the B73 × DE811 F_3 lines. That population was evaluated in only 1 yr at two locations (Beeghly et al., 1997), potentially confounding the results with the effects of the environments. It is also possible that resistance to ECB-ST and increased concentrations of CWCs are correlated in the F_3 lines because both traits were predominantly associated with alleles from DE811. This association may have been maintained in the F_3 lines and dissipated during the additional meioses (and recombination) that occurred during the development of RILs. The RILs of B73 × B52 were evaluated in the same environments as the RILs of B73 × DE811 during 1998, reducing the effect of environment on comparisons between those populations.

Ten QTL were detected for ECB-ST adjusted for maturity, 9 of which are located within 25 cM of QTL for at least one CWC (Krakowsky et al., 2004, 2005, 2006) (Fig. 1). However, the expected relationship between the signs of the QTL effects for the two traits was only observed in about half the instances where QTL were linked; an allele from DE811 that was associated with resistance to ECB-ST adjusted for maturity was as likely to be associated with increased concentrations of CWCs as decreased concentrations of CWCs. There was also no apparent correlation between the size of the additive effects for linked QTL for ECB-ST adjusted for maturity and CWC concentrations. These factors may explain the absence of significant overall genotypic correlations between ECB-ST adjusted for maturity and CWC concentrations in this population. Only three of the nine QTL for ECB-ST in the RILs of B73 × B52 were linked (within 25 cM) to QTL for ECB-ST adjusted for maturity in the RILs of B73 × DE811 (Krakowsky et al., 2004; Cardinal and Lee, 2005). Two of those QTL are on chromosome 2, and the patterns in the RILs of B73 × B52 are similar to those observed herein; at *umc8*, the allele(s) from B73 is associated with increased ECB-ST and stalk ADF concentrations, while at *umc4* the allele(s) from B73 is associated with increased ECB-ST and reduced stalk ADF concentrations. The third QTL is on chromosome 9 and is not associated with QTL for ADF. The lack of consistent localization of QTL across the populations may indicate genetic heterogeneity for resistance to ECB-ST (Krakowsky et al., 2004).

The clustering of QTL for ECB-ST adjusted for maturity and CWC concentrations raises the question of whether the QTL for the two traits are linked by random chance or if the QTL represent pleiotropic genes affecting both traits. Five QTL each for sheath and stalk NDF adjusted were linked to QTL for ECB-ST adjusted for maturity, with no clear association between NDF concentrations and resistance to ECB-ST. For sheath NDF adjusted, the allele from one parent was associated with resistance to tunneling and increased CWC concentrations in only two cases, and for stalk NDF the expected relationship was observed in three cases. Three QTL for ECB-ST adjusted for maturity were linked to QTL for sheath ADF, with the allele from one parent associated with resistance to stalk tunneling and increased concentrations of sheath ADF in two cases, while six QTL for ECB-ST adjusted for maturity were linked to QTL for stalk ADF, with the expected relationship observed in three cases. The NDF adjusted may be uncorrelated or even positively correlated with ECB-ST, because it represents the relatively digestible hemicellulosic fraction of the cell wall; in the sheath, NDF adjusted is higher in B73 than in DE811 (Krakowsky et al., 2006). In the case of ADF, however, a pattern does appear to be present: eight of the nine QTL for ECB-ST adjusted for maturity were linked to QTL for sheath or stalk ADF, and the alleles from DE811 were associated with increased ADF concentrations at all of these QTL. While this pattern could indicate that ECB-ST adjusted for maturity is negatively correlated with ADF in inbred DE811 (i.e., increased concentrations of ADF are correlated with decreased levels of stalk tunneling), it would also hold that the opposite is true for inbred B73.

The proportions of the genotypic covariances associated with linked QTL for ECB-ST adjusted for maturity and sheath and stalk NDF adjusted and ADF are listed in Table 1. While the genetic correlations between the traits indicate relatively small genotypic covariances,
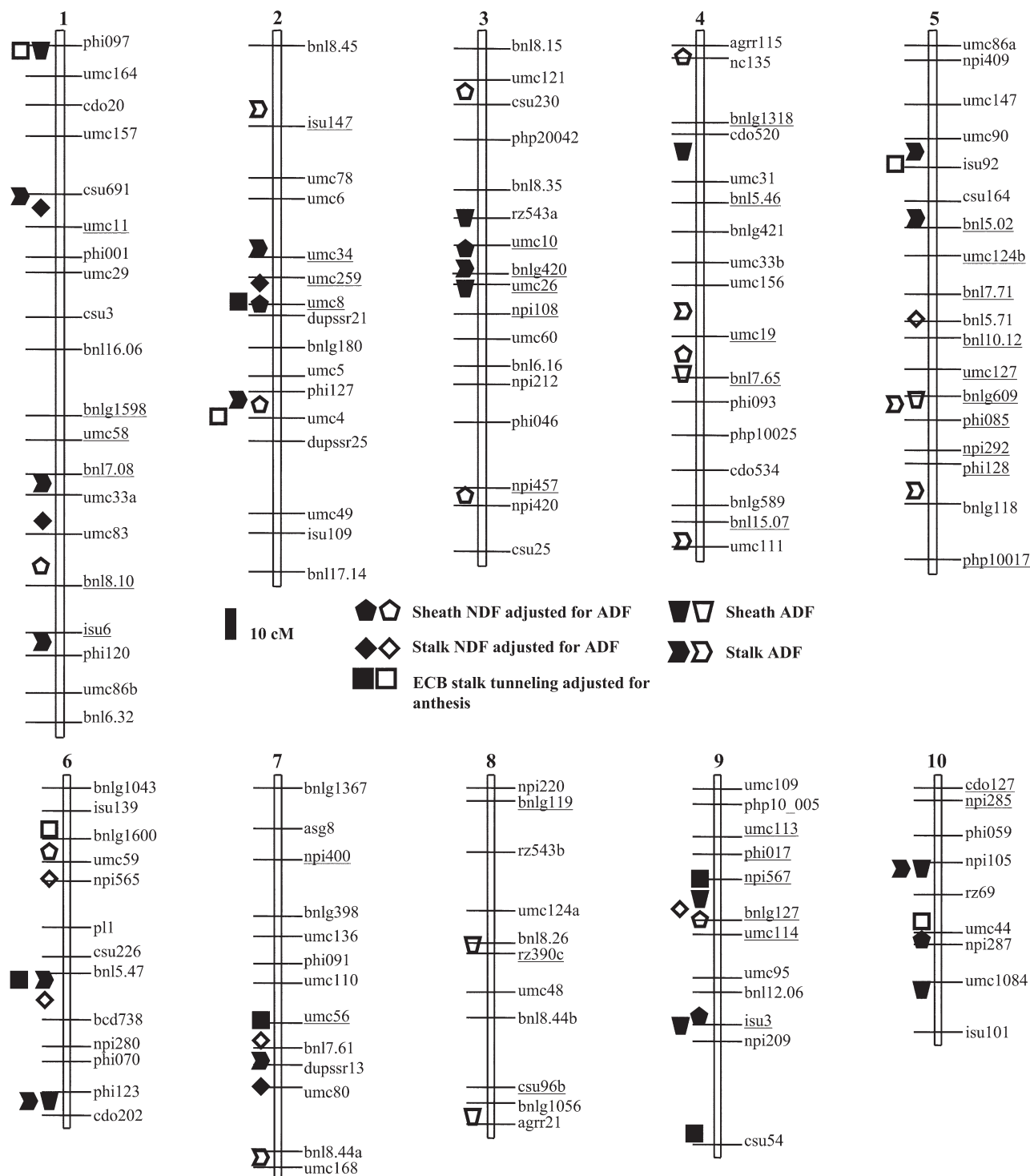
Figure 1. Linkage map of B73 × DE811 recombinant inbred lines and location of quantitative trait loci (QTL) for cell wall components and stalk tunneling. Underlined loci exhibited segregation distortion ($p < 0.05$). Solid shapes denote QTL for which the allele from DE811 is associated with an increase in the trait, while outlined shapes denote QTL for which the allele from B73 is associated with an increase in the trait.

dissection of those covariances can provide information on the genetic basis of a relationship between two traits that would not otherwise be apparent. The total genetic variances explained by the CWC QTL in Table 1 are relatively small as compared to those for the full models reported previously (Krakowsky et al., 2005, 2006), due to the smaller number of QTL in the models herein.

The proportion of the genotypic covariance between specific CWCs and ECB-ST explained by mapped QTL was greater than 1 or less than –1 for several stalk and sheath NDF adjusted QTL. This is possible because covariances can be negative or positive, and covariances of equal magnitude, but opposite sign, will cancel each other out when summed across loci in the total genotypic covariance.

Table 1. Proportion of total genotypic covariances between, and total genotypic variances of, stalk tunneling by the European corn borer (ECB-ST) adjusted for maturity and cell wall component (CWC) concentrations.

Trait	Marker	Bin	Proportion of total genotypic covariance between traits[†]	Proportion of total genotypic variance for ECB-ST adjusted for maturity	Proportion of total genotypic variance for CWC
Sheath neutral detergent fiber (NDF) adjusted for sheath acid detergent fiber (ADF)	umc8	2.04-5	1.17	0.17	0.01
	umc4	2.08	0.57	0.04	0.01
	bnlg1600	6.01	1.13	0.06	0.04
	npi567	9.02-3	−0.74	0.05	0.01
	npi287	10.06	−1.83	0.09	0.07
	Total		0.30	0.41	0.14
Sheath ADF	phi097	1.01	−0.32	0.03	0.03
	npi567	9.02-3	0.36	0.04	0.03
	npi105	10.03	−0.28	0.06	0.01
	umc1084	10.07	−0.60	0.03	0.12
	Total		−0.85	0.16	0.18
Stalk NDF adjusted for stalk ADF	umc8	2.04-5	1.18	0.11	0.08
	bnlg1600	6.01	0.53	0.11	0.01
	bnl5.47	6.05	−0.49	0.05	0.03
	bnl7.61	7.04	−0.11	0.02	0.00
	npi567	9.02-3	−0.26	0.05	0.00
	Total		0.84	0.33	0.12
Stalk ADF	umc8	2.04-5	0.33	0.21	0.00
	phi127	2.08	−0.44	0.02	0.03
	isu92	5.02	−0.48	0.01	0.06
	bnl5.47	6.05	0.12	0.03	0.00
	bnl7.61	7.04	0.29	0.00	0.04
	npi105	10.03	−0.64	0.09	0.02
	Total		−0.82	0.36	0.14

[†]The total proportion explained is expected to be a positive value between 0 and 1. Negative values indicate that the model does not include all markers associated with positive correlations between the CWC and ECB-ST adjusted for maturity.

There were five significant loci for sheath NDF adjusted, and while only a small proportion of the total genotypic covariance between NDF adjusted and ECB-ST adjusted for maturity is accounted for, three of the loci each account for relatively large proportions of the genotypic covariance. The proportions of genotypic covariance between sheath and stalk ADF and ECB-ST adjusted for maturity are not greater than 1 or less than −1 for any individual locus, but the presence of proportionally large positive and negative associations could explain the relatively small genotypic covariances between the traits. The significance of negative genetic associations between ECB-ST adjusted for maturity and ADF at individual loci may be overwhelmed in the analysis across loci due to positive and negative associations at different loci, and the fact that the associations are significant for less than half of the loci for any particular trait (i.e., there are more QTL for CWCs that are not linked to QTL for ECB-ST adjusted for maturity than are linked). That a subset of the QTL for CWC concentrations is associated with resistance to ECB-ST may also explain the results of selection in the BS9(CB) C2 and WFISILO and WFISIHI populations. Selection for lower CWC concentrations in BS9(CB) C2-Lo and WFISILO resulted in increased ECB-ST, but selection for higher CWC concentrations in BS9(CB) C2-Hi and WFISIHI did not consistently result in decreased ECB-ST (Ostrander and Coors, 1997). Selection for lower CWC concentrations probably reduced concentrations of all CWCs, but selection for increased CWCs may not have significantly altered the allelic frequencies at the subset of loci associated with increased CWC concentrations and resistance to ECB-ST.

The hypothesis that resistance to ECB-ST in DE811 is at least partially conferred by concentrations of CWCs in the sheath and stalk is supported by the data presented in Table 1, particularly by the associations between QTL for ECB-ST adjusted for maturity and sheath and stalk ADF. However, the B73 allele(s) was associated with resistance to stalk tunneling, but not with increased concentrations of ADF at several of the QTL for ECB-ST adjusted for maturity. One possible explanation is related to temporal changes in the starch concentrations in the stalk. Lübberstedt et al. (1998)

report the locations of QTL for starch concentration in four different populations, and these QTL are within 25 cM of the ECB-ST adjusted for maturity QTL on chromosomes 2 (*umc8*), 6 (*bnl5.47*), 7 (*umc56*), and 10 (*umc44*). The B73 allele is associated with resistance at those loci (with the exception of the last locus), which could indicate that starch concentrations in the stalk are negatively correlated with resistance to stalk tunneling. It is possible that the decrease in starch concentration in the B73 stalk is greater than in the DE811 stalk between flowering and maturity. The CWC concentrations presented by Krakowsky et al. (2005, 2006) were measured 1 wk after flowering. Lundvall et al. (1994) report in vitro dry matter digestibility (IVDMD) for B73 and for B68 and B37 (which comprise about 75% of the parentage of DE811) near flowering and near physiological maturity. The decrease in IVDMD of the stalk of B73 is greater over the course of the season than that of B68 or B37, likely due to a greater decline in starch concentrations in B73 stalks. Lower starch concentrations may be correlated with decreased ECB-ST through a reduction in available energy.

Genotypic correlations between ECB-ST adjusted for maturity and concentrations of CWCs were not significant in the RILs of B73 × DE811, but negative correlations between stalk and sheath ADF and ECB-ST adjusted for maturity are apparent at several QTL for resistance to ECB-ST. Clustering of QTL for different CWCs complicates calculation of genotypic correlations between specific CWCs and resistance to ECB-ST. The presence of positive and negative associations between different CWCs and ECB-ST adjusted for maturity at the same locus, and between the same CWC and ECB-ST adjusted for maturity at different loci, likely masks the correlations between CWC concentrations and resistance to ECB-ST. The lack of genetic variability for lignin in this population precludes an association with ECB-ST, and stalk and sheath NDF adjusted, which are largely composed of hemicellulose, may be positively correlated with ECB-ST. While phenotypic selection for increased sheath and stalk ADF concentrations may not provide linear increases in resistance, marker–assisted selection for DE811 alleles at *phi097*, *umc4*, *isu92*, and *umc44* could facilitate the transfer of partial resistance to ECB-ST into a B73 background. Starch and CWC concentrations as the plant approaches physiological maturity may also warrant examination, both to expand our understanding of the mechanisms of resistance and as potential resistance factors for use in development of new germplasm.

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