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Full Length Research Paper

Genetic loci mapping for ear axis weight using recombinant inbred line (RIL) population under different nitrogen regimes in maize

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Ear axis weight (EAW) is one of the important agronomic traits in maize (*Zea mays* L.), related to yield. To understand its genetic basis, a recombinant inbred line (RIL) population, derived from the cross Mo17 × Huangzao4, was used for quantitative trait locus mapping (QTL) for EAW under high and low nitrogen (N) regimes. The results showed that a total of three QTLs were mapped on chromosomes 2 (two) and 4 (one) under the two N regimes, which could explain phenotypic variances from 4.76 to 7.12%. They were near to their linked markers, with mapping interval of 0.2 to 1.0 cM. The two loci on chromosome 2 (bin 2.09) made EAW increase due to positive additive effects, while the other locus on chromosome 4 (bin 4.08) made EAW decrease to some extent, owing to negative additive effects. These results are beneficial for understanding the genetic basis of KNE and developing marker-assisted selection in maize breeding project.

Key words: Maize (*Zea mays* L.), ear axis weight, quantitative trait locus, recombinant inbred line, nitrogen.

INTRODUCTION

The previous studies on QTL mapping for maize (*Zea mays* L.) ear-related traits were concentrated on yield per plant (Huang et al., 2010), 100-kernel weight (Guo et al., 2008), ear weight (Sabadin et al., 2008), row number per ear (Lu et al., 2006) and kernel number per row (Li et al., 2010). For ear axis weight (EAW), although it is also an important agronomic trait related to yield in maize breeding program, the study on its genetic basis was hardly reported, except for Wang et al. (2007).

As well known, different parental lines may lead to different results in QTL number, chromosomal location or genetic effect. For example, using X178 and B73 as parental inbred lines, Xiao et al. (2005) identified six QTLs for kernel number per ear on chromosomes 1 (three), 3 (one) and 9 (two), but the report by Agrama et al. (1999), using B73 and G79 as mapping parents, showed that there were four QTLs for the same trait on chromosomes 1 (one), 6 (one), 7 (one) and 9 (one).

Consequently, it is necessary to choose different parental lines to map the QTLs for EAW in maize.

In addition, different environmental conditions can also lead to different results in QTL number, position or genetic effect. Among the ecological conditions influencing maize growth and development, N content in soil is very important. Currently, most maize in the world are grown under N-deficient conditions (Ribaut et al., 2007). From literature, different N conditions have been frequently used to detect QTLs in plant such as rice (*Oryza sativa* L.) (Lian et al., 2005), maize (Liu et al., 2008) and wheat (*Triticum aestivum* L.) (An et al., 2006), but the studies on using different N environments to map QTL for maize EAW were not reported. Accordingly, it is meaningful that different N regimes are used for QTL mapping affecting EAW in maize.

Therefore, the two parental lines Mo17 and Huangzao4 were used as mapping parents, based on their RIL population; the QTL(s) controlling EAW were identified under two N regimes. The objectives here were to (1) realize the genetic basis of EAW more clearly in maize; (2) find some molecular markers co-segregated with the

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Table 1. The phenotypic values of parental lines and F_1 for EAW.

N regime	M017		HZ4		F_1 hybrid	
	Mean (g)	SD	Mean (g)	SD	Mean (g)	SD
HNR	8.31	1.80	9.97	1.36	26.09	0.65
LNR	6.75	2.09	7.06	0.85	23.15	1.44

Table 2. Descriptive statistics of RIL population for EAW under two N regimes.

N regime	Range (g)	Minimum (g)	Maximum (g)	Mean (g)	[a]SD	[b]CV (%)	Skewness	Kurtosis
HNR	13.61	2.92	16.53	9.96	2.53	25.40	0.16	0.01
LNR	11.31	3.86	15.17	9.39	2.24	23.86	0.23	-0.17

[a]SD, Standard deviation; [b]CV, coefficient of variation.

genetic loci controlling EAW which can be used for MAS in maize.

MATERIALS AND METHODS

Plant materials

The experimental materials involved in this study included maize inbred lines Mo17 (high EAW) and Huangzao4 (low EAW) as parents, their F_1 hybrid and RIL population consisting of 239 RILs. Mo17 and Huangzao4 are the representative lines of Lancaster and Tangsipingtou heterotic groups, respectively, the F_1 hybrid and RIL population were derived from the cross between the two parental lines.

Experiment measurements and phenotypic observation

Parental lines, F_1 and the RIL population were sown in a randomized complete block design with six replicates at the experiment farm of Nanchong Institute of Agricultural Sciences, Nanchong City, P. R. China, with single-plant planting and 15 plants per replicate, of which three replicates were under high N regime (HNR) by appending urea 300 kg/ha and the other three replicates were under low N regime (LNR) with no appended N fertilizer. The average contents of total N and alkaline hydrolysis N in 30-cm-depth soil were 0.092 and 0.000056%, respectively.

At the time of harvest, eight plants in the middle of every replicate were individually investigated for the trait EAW. Based on the investigated data of the RIL population, SPSS11.5 software (www.spss.com) was used to perform descriptive statistics, analysis of variance (ANOVA) and correlation analysis for the trait EAW.

QTL detection

Based on the data of EAW in the RIL population and the established genetic map consisting of 100 SSR markers and covering 1421.5 centiMorgan (cM) of mapping distance (Liu et al., 2009), the QTL(s) controlling EAW under two N regimes were severally analyzed by composite interval mapping (CIM) method of Windows QTL Cartographer 2.5 software (Wang et al., 2010), scanning interval of 2 cM between markers and putative QTLs with a window size of 10 cM. The LOD (log10 of odds ratio) threshold value for the QTL significance was determined by 1000-time permutation test ($\alpha = 0.05$) (Doerge and Churchill 1996); cofactors used for calculation of CIM were selected by the program using forward stepwise regression, LOD curves were created by scanning all linkage groups, the QTLs with a LOD value greater than the threshold value was presented and their position, genetic effects and percentage of phenotypic variation were estimated at the significant LOD peak in the region. The QTLs identified under the two N regimes were mapped with Mapchart 2.1 software (Voorrips, 2002).

RESULTS AND DISCUSSION

Statistics analyses

Statistic results indicated that the tested lines presented variation in EAW (Table 1) for the three lines including parents and F_1 hybrid; F_1 hybrid had the highest values under both N regimes because of heterosis, followed by Huangzao4. Moreover, all the three lines possessed higher values under HNR than those under LNR. For the RIL population, the results of the descriptive statistics are listed in Table 2. Among the eight statistic parameters, all of them showed higher values under HNR than those under LNR except for minimum and skewness. The results of ANOVA of the RIL population on EAW under two N regimes are demonstrated in Table 3. Different lines of the RIL population provided differences at 0.01 probability level under any one of the two N regimes. Nevertheless, the two-group data of the population obtained under two N environments presented positive relation at 0.01 probability level ($r = 0.846$). In addition, from the frequency distribution graphs (Figures 1a, b), the data of the RIL population under both N regimes could well agree with normal distribution, which meant that the trait EAW is a quantitative trait and is controlled by multiple genes in maize.

QTL identification

Permutation test indicated that the LOD threshold values

Table 3. ANOVA of the RIL population on EAW under two N regimes.

N regime	Variation source	Sum of squares	[a]df	Mean square	F	Significance
HNR	Between groups	4499.14	234	19.23	7.98**	<0.01
	Within groups	1132.92	470	2.41		
	Total	5632.06	704			
LNR	Between groups	3533.61	234	15.10	5.53**	<0.01
	Within groups	1284.42	470	2.73		
	Total	4818.02	704			

[a]There were four missing values among the RIL population consisting of 239 RILs; ** significant difference at 0.01 probability level.

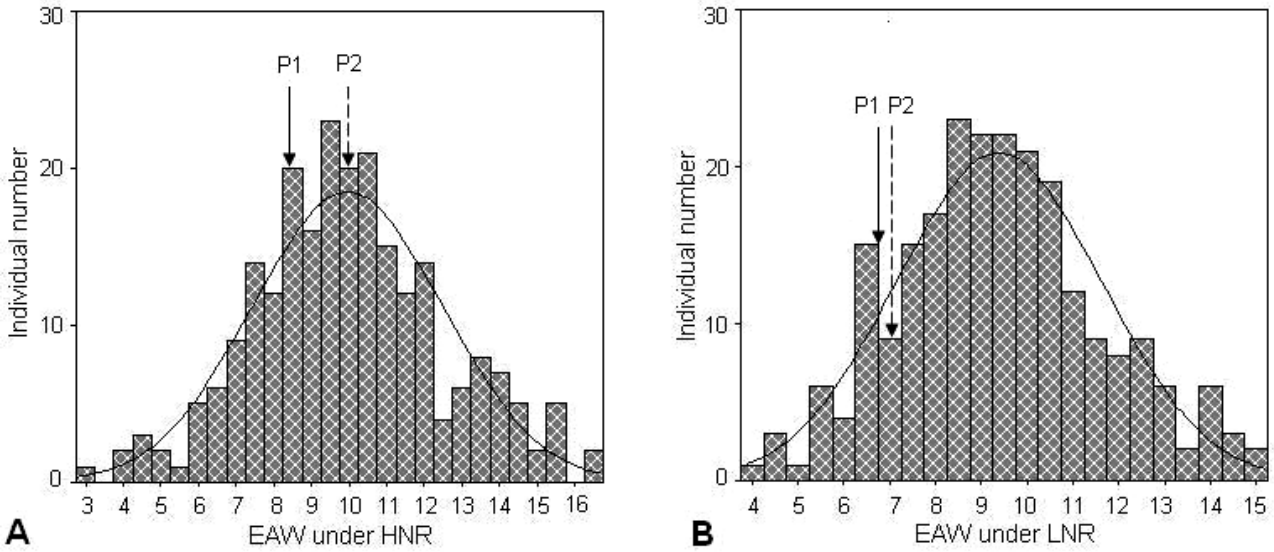

Figure 1. Frequency distribution graphs of the RIL population for EAW under two N regimes. A, EAW under HNR; B, EAW under LNR. The means of parental lines are indicated by arrows, P1 for Mo17 and P2 for Huangzao4.

Table 4. Positions and effects of the QTLs associated with EAW identified under two N regimes.

N regime	QTL	Chromosome	The proximal markers	[a]Mapping interval (cM)	[b]LOD	[c]R^2 (%)	Additive effect
HNR	*Qeaw1*	2	Bnlg1520 (bin2.09)	1.0	3.70	6.81	0.67
LNR	*Qeaw2*	2	Umc1736 (bin2.09)	0.9	3.75	7.12	0.60
	Qeaw3	4	Umc2188 (bin4.08)	0.2	2.78	4.76	-0.49

[a]The mapping interval between QTL and linker marker; [B]the $\log_{10}$ of odds ratio; [C]percentage of phenotypic variation explained by QTL.

of QTL significance associated with EAW should be set at 2.61 and 2.51 under HNR and LNR, respectively. Based on the LOD values, a total of three QTLs were detected under both N regimes (Table 4 and Figure 2). The QTL identified under HNR (named *Qeaw1*) was located on chromosome 2, linked with Bnlg1520, with a mapping interval of 1.0 cM and this locus could explain 6.81% of the phenotypic variance and made EAW increase (0.67 g) due to additive effect. For the two QTLs mapped under LNR, one was on chromosome 2 (named *Qeaw2*), while the other was on chromosome 4 (named *Qeaw3*). With 0.9 and 0.2 cM near to their linked markers Umc1736 and Umc2188, respectively, they could account for 7.12 and 4.76% of the phenotypic variance, respectively. The two genetic loci identified under LNR presented contrary genetic effects due to different additive effects; *Qeaw2* and *Qeaw3* could make EAW increase and decrease, respectively.

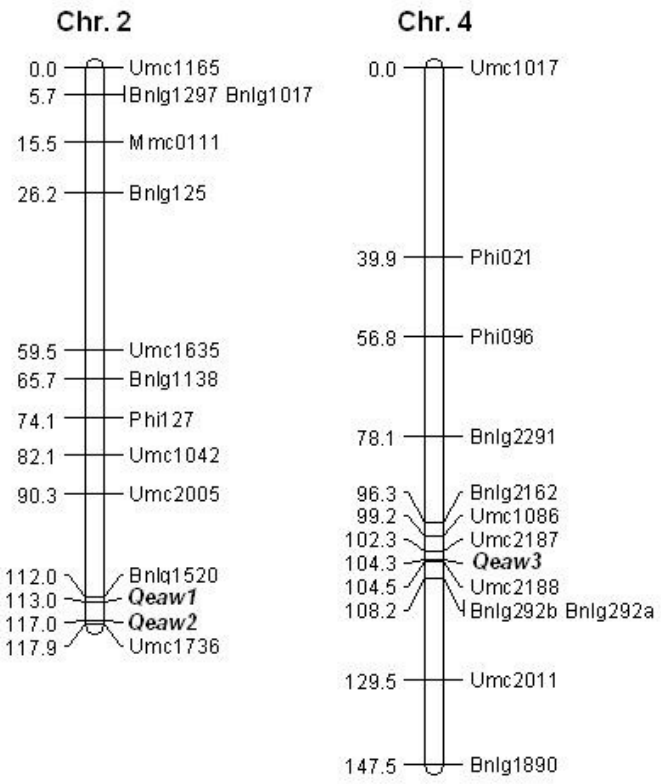

Figure 2. Chromosomal positions of the QTLs for EAW identified using the RIL population derived from Mo17× Huangzao4 under two N regimes. *Qeaw1* and *Qeaw2* were detected under HNR, while *Qeaw3* was identified under LNR.

Table 5. The QTLs for EAW were reported in different studies in maize.

Reference	Parental line	Population	Environment	QTL number (chromosomal position)
Wang et al. (2007)	Lo1067 and Yi72	F_2	Two water regimes	3 (one on chromosome 1 and two on chromosome 2)
This study	Mo17 and Huangzao4	RIL	Two N regimes	3 (two on chromosome 2 and one on chromosome 4)

The QTLs for EAW were also reported by Wang et al. (2007), but our study was different from theirs in many aspects and the main differences are listed in Table 5. From QTL position, the QTLs identified in this study were obviously different from the previous, so they belonged to new loci associated with EAW in maize. It can be mentioned that the segregating population in our experiment was immortal due to the presence of homologous lines and can be used in different regions and time. However, this kind of population in Wang et al. (2007) was temporary and cannot be utilized again, because of no continued plants use for further phenotypic and genetic analysis (Pilet et al., 2001). Additionally, the environmental conditions used for QTL mapping in the report by Wang et al. (2007) were different water-content in the soil, whereas in our experiment, two N regimes were employed in QTL mapping. It is important to note that different N conditions were first designed to map QTL for EAW.

Conclusions

A RIL population, derived from the two parental lines Mo17 and Huangzao4, was used to map the QTLs associated with EAW under two N regimes. The results showed that a total of three QTLs were mapped on chromosomes 2 (*Qeaw1* and *Qeaw2*) and 4 (*Qeaw*3) which could explain the phenotypic variances from 4.76 to 7.12%. They were near to their linked markers Bnlg1520, Umc1736 and Umc2188, respectively, with mapping interval 0.2 to 1.0 cM. The two loci on chromo-

some 2 (bin2.09) provided positive additive effects, while the locus on chromosome 4 (bin4.08) possessed negative additive effects. These results are beneficial for understanding the genetic basis of KNE and developing MAS in maize breeding project.

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