

Full Length Research Paper

QTL mapping for ear length and ear diameter under different nitrogen regimes in maize

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Accepted 15 February, 2010

Ear length (EL) and ear diameter (ED) are two of the most important traits in maize (*Zea mays* L.), related to yield and plant morphology. In this study, an F₉ recombinant inbred line (RIL) population was used to identify the quantitative trait loci (QTLs) controlling EL and ED under two nitrogen (N) regimes. As a result, 3 QTLs were detected under N rich environment, 1 for EL on chromosome 1 and 2 for ED on chromosome 9. Under N stress environment, 4 QTLs were identified, 1 for EL on chromosome 1 and 3 for ED on chromosome 4 and 9. The phenotypic variances explained by these QTLs ranged from 5.68% to 9.86%, their additive effects were from -0.74 to 0.16. These results were beneficial for realizing the genetic basis of EL and ED and carrying out marker-assisted selection in maize.

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

INTRODUCTION

Nitrogen (N) stress condition presents a major source of yield loss in maize (*Zea mays* L.) (Pingali and Pandey, 2001). Currently, most maize in developing countries is produced under N-deficient conditions because of low N use efficiency, limited availability of fertilizer, or low purchasing power of farmers (Bänziger et al., 1997; Ribaut et al., 2007). N deficiency will affect maize growth and development, and the ear-relevant characters including ear length (EL) and ear diameter (ED) will be affected severely. EL and ED are two important traits in maize breeding program, but at present, the maize germplasm with long and large ears are quite lacking. One of the effective ways to resolve this problem is to breed long-ear and large-ear varieties, whereas, the conventional

breeding method is much time-consuming, and the available resources are very limited. An alternative solution is to utilize the elite genes to improve the two traits, and the basic work is to realize the genetic basis of EL and ED. Quantitative trait locus (QTL) mapping is an efficient approach to realize their genetic basis up to now, some QTLs controlling EL and ED have been reported in maize (Xiang et al., 2001; Tang et al., 2007; Liu et al., 2007; Lu et al., 2008; Xie et al., 2008; Sabadin et al., 2008). But, different studies showed differences in several aspects including number, location and effect of QTL, which may be explained by different mapping parents, segregation population or experimental environments.

N environment of soil is a very important factor influencing gene expression, same gene will probable present expression variation under different N supplies, thus, different N regimes have frequently been used for QTL mapping in plant such as rice (*Oryza sativa* L.) (Shan et al., 2005), maize (Liu et al., 2008) and wheat (*Triticum aestivum* L.) (An et al., 2006). For example, Ribaut et al. (2007) detected 8 QTLs for maize anthesis-silking interval under N stress condition, while under

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Abbreviations: QTL, Quantitative trait locus; RIL, recombinant inbred line; EL, ear length; ED, ear diameter; N, nitrogen; NRE, nitrogen rich environment; NSE, nitrogen stress environment; LOD, log₁₀ of odds ratio; MAS, marker-assisted selection.

normal N environment, only 3 QTLs affecting the trait were identified. Regarding the two traits EL and ED of maize, Liu et al. (2007) mapped 4 and 3 QTLs under N normal environment respectively, but under N stress condition, 3 and 4 QTLs were found, respectively. Thus, it was necessary and significant that different N regimes were used for identified QTLs for EL and ED in maize.

Moreover, the previous segregation populations were focused on F_2 in QTL mapping in maize (Yang et al., 2005; Sabadin et al., 2008), this type of population is temporary and could not be reused, because no continued plants were used for DNA extraction and phenotypic investigation, while, recombinant inbred line (RIL) population could be reused again and again duo to homogenous individuals (Pilet et al., 2001). To date, there were hardly few studies on QTL mapping for EL and ED using RIL population in maize.

In this present study, an F_9 RIL population and two N regimes were used to map the QTLs for EL and ED in maize. The objectives are to (1) identify and compare the QTLs controlling EL and ED under different N conditions, and (2) look for the marker loci used for marker-assisted selection in maize breeding.

MATERIALS AND METHOD

Plant materials

The experimental materials included parental lines Mo17 and Huangzao4, F_1 and an F_9 RIL population consisting of 239 F_9 RILs. Mo17 and Huangzao4 belong to Lancaster and Tangsipingtou heterotic groups, respectively; the RIL population was derived from the cross between Mo17 and Huangzao4.

Experimental design

All the 242 lines above were sown in a complete randomized design with six replicates and 15 plants each replicate for every line at Maize Research Institute, Shanxi Academy of Agricultural Sciences, Xinzhou City, P. R. China, three replicates under N rich environment (NRE) applied urea 300 kg/ha, the other three replicates under N stress environment (NSE) with no N fertile applied. The basal N level of the experimental field were 920 mg/kg of total N and 56 mg/kg alkali-hydrolyzed N in the top 30 cm of soil, and thus, N stress and N rich environments would be formed as long as no N fertilizer and 300 kg/ha urea were applied into the soil, respectively.

Phenotypic analyses

At harvest stage, the middle eight plants of each line of every replicate were individually investigated on the two traits EL and ED. Based on the investigated data, the average values of EL and ED of every line under same N condition were calculated and used for statistical analysis.

QTL mapping

Based on the data of EL and ED of the RIL population and the previous genetic map established by Liu et al. (2009), consisting of

100 microsatellite sites on ten chromosomes and covering 1421.5 cM of mapping distance with an average of 14.2 cM between two adjacent markers, the QTL(s) controlling EL and ED were analyzed by Composite Interval Mapping (CIM) method of Windows QTL Cartographer 2.5 software (Wang et al., 2007), scanning interval of 1 cM between markers and putative QTLs with a window size of 10 set by forward stepwise regression. The threshold value for the QTL significance was determined by 1000-time permutation test ($\alpha = 0.05$) (Churchill and Doerge, 1994; Doerge and Churchill, 1996), cofactors used for calculation of CIM were selected by the program using forward stepwise regression, \log_{10} of odds ratio (LOD) curves were created by scanning every 1 cM of all possible linkage groups, the QTLs with a LOD value greater than the threshold value will be presented, and their position, genetic effects and percentage of phenotypic variation were estimated at the significant LOD peak in the region. The QTLs identified were mapped using Map chart 2.1 software (Voorrips, 2002).

RESULTS

Phenotypic observation and statistic analysis

The investigations on EL and ED indicated that the tested lines appeared variation. The two parents showed significant differences under the two N conditions (Table 1). The ear of Mo17 was up to 13.7 and 13.1 cm under NRE and NSE, respectively, much higher than Huangzao4. But, its ED was less than Huangzao4 under same N condition. F_1 presented higher values of ED and EL than those of the parents under same N environment. The RIL population demonstrated high variation for EL and ED under different N regimes. But to be noticed, the average values of EL or ED obtained under NRE was not significant higher than the corresponding values under NSE. This suggested that too much N fertile in soil would not contribute to the growth and development of maize ear. Moreover, according to the data in Table 1, it was found that the four group data in the RIL population, including EL under NRE, EL under NSE, ED under NRE and ED under NSE, could well agree with normal distribution.

QTL mapping

Permutation test for EL under NRE, EL under NSE, ED under NRE and ED under NSE, showed that their threshold values for the QTL significance were 2.53, 3.07, 2.74 and 2.22, respectively. According to the criterion thresholds, under NRE, 3 QTLs were detected by mapping analysis, including one controlling EL on chromosome 1 and 2 controlling ED on chromosome 9 (Figure 1). The linked markers to them were Bnl439, Phi061 and Nc134, respectively. Under NSE, total 4 QTLs were mapped on chromosome 1, 4 and 9, including 1 for EL and 3 for ED. To be mentioned, both *Qednr9-2* and *Qedns9-1* were quite near to their same linked marker Nc134, with 0 cM of genetic interval. In addition, *Qednr9-1* was also very close to marker Phi061, less than 1 cM of genetic distance between them. These QTLs could explain phenotypic variances from 5.68% to

Table 1. Descriptive statistics of EL and ED of parents, F₁ and population under two N regimes.

Traits	Conditions	Mean of Mo17	Mean of Huangzao4	Mean of F ₁	Population					
					Minimum	Maximum	Mean	^a SD	Skewness	Kurtosis
EL	NRE	13.7	10.4	18.8	7.6	18.6	12.4	1.896	0.293	0.474
	NSE	13.1	9.0	18.0	8.0	18.3	12.2	1.811	0.386	0.434
ED	NRE	3.1	4.4	4.7	2.9	4.7	3.8	0.339	-0.102	-0.283
	NSE	3.2	4.2	4.8	2.9	4.7	3.9	0.320	-0.135	-0.103

^aSD, standard deviation.**Table 2.** The QTLs for EL and ED detected under NRE and NSE.

Traits	Conditions	QTLs	Chromosome	Interval	Position (cm)	LOD	^a R ²	^b A
EL	NRE	<i>Qelnr1-1</i>	1	Bnlg1178-Bnlg439	38.0	3.37	9.86	-0.74
	NSE	<i>Qelns1-1</i>	1	Bnlg1178-Bnlg439	36.0	3.17	9.69	-0.70
ED	NRE	<i>Qednr9-1</i>	9	Phi022-Phi061	48.8	5.30	8.70	0.16
		<i>Qednr9-2</i>	9	Nc134-Phi067	57.7	4.96	8.70	0.16
	NSE	<i>Qedns4-1</i>	4	Phi096-Bnlg2291	68.8	2.33	5.68	0.15
		<i>Qedns4-2</i>	4	Bnlg292b-Umc2011	108.2	3.76	6.08	-0.14
		<i>Qedns9-1</i>	9	Nc134-Phi067	57.7	3.90	6.89	0.14

^aR², the percentage of phenotypic variance explained by the corresponding QTL; ^bA, additive effect.

9.86% (Table 2). Moreover, according to the additive effects of these QTLs (Table 2), it was concluded that *Qelnr1-1* and *Qelns1-1* from Huangzao4 could make EL decrease 0.74 and 0.70 cm, respectively, *Qedns4-2* from Huangzao4 could make ED decrease 0.14 cm, while, the other four QTLs (*Qednr9-1*, *Qednr9-2*, *Qedns4-1* and *Qedns9-1*) from Mo17 could make trait ED increase from 0.14 cm to 0.16 cm, duo to positive additive effects (Table 2).

DISCUSSION

N deficiency can severely affect many metabolic pathways and physiological progresses in maize (Ribaut et al., 2007), and low growth rate for ear is one of the most serious consequences. To realize the genetic basis of the two important agronomic traits EL and ED, in this study, a RIL population, derived from the cross between Mo17 and Huangzao4, was used to identify the QTLs controlling EL and ED under different N regimes. As a result, for trait EL, two QTLs for EL *Qelnr1-1* and *Qelns1-1* from Huangzao4 were detected on chromosome 1 under NRE and NSE respectively, and could make EL decrease 0.74 and 0.70 cm respectively, due to negative additive effects. For trait ED, total 5 QTLs were mapped on chromosome 4 and 9, the two including *Qednr9-1* and *Qednr9-2* from Mo17 were identified under NRE, and could make ED increase duo to positive additive effects; the three including *Qedns4-1*, *Qedns9-1* and *Qedns4-2*

were detected under NSE, of which *Qedns4-1* and *Qedns9-1* from Mo17 could make ED increase 0.15 cm and 0.14 cm, respectively; while, *Qedns4-2* from Huangzao4 could decrease ED about 0.14 cm. But, to be noticed, the two QTLs *Qelnr1-1* and *Qelns1-1* on chromosome 1 were very near to each other, with only 2.0 cM of map distance between them, this suggested that they were probably same QTL and the gene harbored by the locus could express under both the two N conditions, the result was similar to the two QTLs *Qedns9-1* and *Qednr9-2*. Compare to the report by Liu et al. (2007), similar N environments were designed to map QTLs for EL and ED, but our studies were different from theirs in several aspects, and the main differences were displayed in Table 3. To be mentioned, all the loci reported here were different from those reported by them.

Besides Liu et al. (2007), some other parents and environments were also used to analyze the QTL controlling EL and ED (Xiang et al., 2001; Tang et al., 2007; Lu et al., 2008; Xie et al., 2008; Sabadin et al., 2008). But, the QTL number, location and effects showed differences in different studies, this may be explained by the differences of mapping parents, segregation population, molecular marker or experimental environments. To be mentioned, the previous segregation populations were focused on F₂ in QTL mapping for EL or ED (Liu et al., 2007; Xiang et al., 2001; Lu et al., 2008; Sabadin et al., 2008), this kind of population is temporary, and could not be reused due to no continued plants, for it could not supply continued plants for phenotypic and genetic analy-

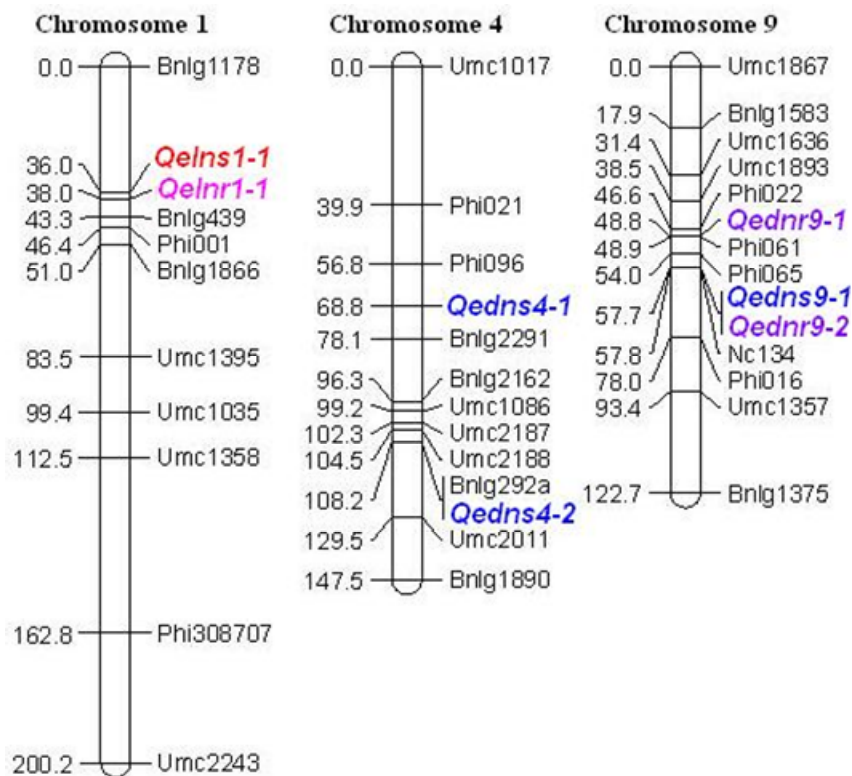


Figure 1. Genetic map of the QTLs controlling EL and ED identified under NRE and NSE.

Table 3. QTLs for EL and ED identified under different N environments.

References	Parents	Populations	Traits	Environments	Chromosomal position of linked markers (bin)
Liu et al., 2007	HuangC Xu178	F _{2:3}	EL	N normal	1.04; 3.04; 6.00; 9.04
				N stress	1.04; 1.05; 8.06
			ED	N normal	2.04; 2.05; 9.04
				N stress	1.06; 2.04; 7.03; 7.01
In this study	Mo17, Huangzao4	RIL	EL	N rich	1.03
				N stress	1.03
			ED	N rich	9.03; 9.03
				N stress	4.06; 4.08/4.09; 9.03

sis (Pilet et al., 2001). Besides, only few molecular markers were used for QTL or gene mapping in some previous reports, for example, only 80 SSR markers were used to construct genetic map in the report by Xiang et al. (2001). Whereas, in this work, an immortal F₉ RIL population consisting of 239 RILs and 100 SSR markers were used for QTL mapping, which could probably improve the reliability of the results.

Furthermore, the QTL for ED, identified under both N environments, was quite near to marker Nc134, with 0 cm of genetic distance between them. This suggested that marker Nc134 could probably be co-segregated with the

gene controlling ED within *Qednr9-2* or *Qedns9-1*; it could be used for MAS in maize breeding program, similar to the previous reports (Dußle et al., 2003; Wu et al., 2007). In addition, the QTL *Qednr9-1* was very close to marker bnlg197, with only 0.3-cM map distance between them, and it could make ED increase 0.16 cm, while, other QTLs identified were far to their linked markers, greater than or equal to 2.0 cM, and thus, it was necessary that more molecular markers were added into these given chromosomal regions, at present, the research work for fine mapping the QTLs controlling EL and ED is in progress based on the constructed RIL

population and genetic map.

ACKNOWLEDGEMENTS

This work was financially assisted by Shanxi Key Subject Wang, Yan Wang, Qi Luo, Xiaopin Wu, (Maize Research Institute, Shanxi Academy of Agricultural Sciences, Xinzhou City, P. R. China) for his technical assistance.

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