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Full Length Research Paper

QTL mapping for controlling anthesis-silking interval based on RIL population in maize

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Nitrogen (N) deficiency will severely affect many metabolic pathways and physiological progresses during maize (*Zea mays* L.) growth and change of anthesis-silking interval (ASI) is one of the most serious consequences. To realize the genetic basis of ASI, a recombinant inbred line (RIL) population consisting of 239 RILs, derived from the cross between Mo17 and Huangzao4, was used to identify the quantitative trait loci (QTLs) controlling ASI under different N environments. As a result, 6 QTLs were detected under high N environment on chromosome 3, 6, 7 and 8 and could explain total 53.67% of phenotypic variance. While, under low N environment, only 3 QTLs were identified on chromosome 6, 7 and 8, and they could account for total 31.87% of phenotypic variance. The two QTLs *Qasihn6-1* and *Qasihn3-1*, identified under high N environment, were quite near to their linked marker Phi077 and Bnlg197, respectively, with less than 1 cM of genetic distance. These results are beneficial for understanding the genetic basis of ASI in maize.

Key words: Maize (*Zea mays* L.), recombinant inbred line (RIL), quantitative trait locus (QTL), anthesis-silking interval (ASI), nitrogen environment.

INTRODUCTION

As is well known, low nitrogen (N) conditions present a major source of yield loss in maize (*Zea mays* L.) (Pingali and Pandey, 2001). 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). N deficiency severely affect many metabolic pathways and physiological progresses (Ribaut et al., 2007) and increasing anthesis-silking interval (ASI) is one of the most serious consequences (Jacobs and Pearson 1991). Too long ASI will probably reduce grain number and further bring about yield loss (Edmeades et al., 1999). One of the effective ways to resolve this problem is to breed short-ASI varieties, whereas, the conventional breeding method is much time-consuming and the available resources are limited. An alternative solution is to utilize the elite genes controlling ASI derived from short-ASI plants to improve the character of long-ASI cultivars and the basic work is to screen short-ASI germplasm and analyze the genetic basis of ASI. QTL mapping is an efficient approach to realize the genetic basis. At present, some QTLs controlling ASI have been reported in maize (Li et al., 2003; Zhang et al., 2004; Szalma et al., 2007; Ribaut et al., 2007; Wu et al., 2008). For example, Li et al. (2003) identified 5 QTLs controlling ASI using F_2 mapping population, on chromosome 1, 2, 3 and 5.

From previous reports on QTL mapping for ASI, it was found that the number, chromosomal loci or effects of QTL were different under different ecological conditions, even related work was finished by same researchers using same genetic map and segregation population. For instance, Zhang et al. (2004) identified one QTL on

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Abbreviations: ASI, Anthesis-silking interval; **RIL,** recombinant inbred line; **QTL,** quantitative trait locus; **N,** nitrogen; **F_2,** second filial; **SSR,** simple sequence repeat; **HNE,** high nitrogen environment; **LNE,** low nitrogen environment; **DS,** days to silking; **DP,** days to pollen; **CIM,** composite interval mapping; **LOD,** log10 of odds ratio; **MAS**, marker-assisted selection; **CV**, coefficient of variation; **SD**, standard deviation .

chromosome 6 under drought stress, but, under well-watered environment, they found other two QTLs distributed on chromosome 2 and 6 using same F_2 population and SSR marker linkage map. This cause may be explained by the environmental regulation of gene expression. Different ecological conditions have been designed to map the QTLs controlling ASI in maize (Ribaut et al., 2007; Wu et al., 2008), of which different water regimes in field were used frequently (Li et al., 2003; Zhang et al., 2004), whereas, N difference in soil was hardly applied in QTL mapping for ASI (Ribaut et al., 2007).

The previous mapping populations for QTL mapping controlling ASI were focused on F_2 and BC (Li et al., 2003; Zhang et al., 2004; Ribaut et al., 2007; Wu et al., 2008), the two types of segregating population were temporary and could not supply continuous plants for extracting genomic DNA and obtaining phenotypic data (Pilet et al., 2001); while, recombinant inbred line (RIL) population is immortal due to homogenous individuals and could be used in different regions and time. At present, this population has been widely used to identify QTLs in many plants (Geffroy et al., 2000; Wan et al., 2006; Ding et al., 2008), but, only few studies on QTL mapping controlling ASI using RIL population were reported in maize to this date.

In the present experiment, QTLs for ASI were mapped and characterized using RIL population under high and low N environments. The main objectives were to: (1) Identify and compare the QTLs controlling ASI under different N conditions, (2) Look for the markers used for marker-assisted selection (MAS) in maize breeding and (3) Offer the primary work for cloning the genes controlling ASI.

MATERIALS AND METHODS

Plant materials

The experimental maize materials included two parental inbred lines Mo17 (long ASI) and Huangzao4 (short ASI), F_1 and a RIL population consisting of 239 F_9 RILs. Mo17 belongs to Lancaster heterotic groups, whereas, Huangzao4 is one of the representative lines in Tansipingtou heterotic groups. The RIL population was derived from the cross between Mo17 and Huangzao4.

Field experiments

All the 242 lines above were sown in a complete randomized design with 6 replicates and 15 plants per replicate, 3 replicates under high N environment (HNE) applied CO(NH2)2 300 kg/ha and three replicates under low N environment (LNE) with no N applied, at the experiment field of Agricultural Research Institute, Nanchong City, P. R. China.

Field measurements and data analysis

At flowering stage, the middle eight plants of every replicate were individually investigated on the two traits, days to silking (DS) and days to pollen (DP). ASI of every plant was calculated using the formula ASI = DS - DP. According to the ASI value of single plant, the average ASI of every line was calculated under same N condition. Based on the data of ASI of the 239 lines, the descriptive statistics and frequency distribution graph of ASI in the RIL population were performed by Statistical Package for the Social Sciences (SPSS) 11.5 software (www.SPSS.com).

QTL mapping

Based on the data of ASI in the RIL population and the established genetic map consisting of 100 SSR markers (Liu et al., 2009), the QTL(s) controlling ASI under two N environments were analyzed by the composite interval mapping (CIM) 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 cM. The number of marker cofactors for the background control was set by forward stepwise regression. The threshold value for the QTL significance was determined by 1000-time permutation test (α = 0.05) (Churchill and Doerge, 1994), cofactors used for calculation of CIM were selected by the program using forward stepwise regression, log10 of odds ratio (LOD) curves were created by scanning every 2 cM of all possible 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 environments were mapped with Mapchart 2.1 software (Voorrips, 2002).

RESULTS

Phenotypic observation and statistic analysis of ASI

The investigation on growth period indicated that the tested lines appeared variations in ASI. Mo17, Huangzao4 and F_1 had 3.0, 1.0 and 0 d of ASI under HNE respectively, whereas, they presented severally 2.7, 0.7 and 0.3 d of ASI under LNE. Both parents had higher ASI under HNE, while, F_1 provided contrary result. The RIL population demonstrated high variation under both environments in ASI (Table 1), ranging from 0 to 6.3 and - 0.7 to 6.7 under HNE and LNE respectively; coefficient of variation (CV) values were up to 53.41 and 57.20% respectively. The frequency distribution graphs obtained under the two environments could well fit the curves of normal distribution (Figures 1 and 2).

QTL identification

Analysis of the genetic map and ASI data by mapping software showed 9 QTLs which were on chromosome 3, 6, 7 and 8, in condition of the LOD threshold set on 2.35 and 2.90 derived from 1000-time permutation test under HNE and LNE, respectively (Figure 3). The six QTLs including *Qasihn3-1*, *Qasihn3-2*, *Qasihn6-1*, *Qasihn7*-1, *Qasihn8-1* and *Qasihn8-2*, were detected under HNE, linked with markers Bnlg197, Umc1659, Phi077, Bnlg1792, Bnlg1863 and Bnlg240, respectively; while, the other three including *Qasiln6-1*, *Qasiln7-1* and *Qasiln8-1* were found under LNE, linked with Phi077, Bnlg1792 and

Table 1. The descriptive statistics of RIL population on ASI under HNE and LNE.

Environments	Minimum	Maximum	SD	CV (%)	Skewness	Kurtosis
HNE	0	6.3	1.33	53.41	0.49	0.09
LNE	- 0.7	6.7	1.43	57.20	0.58	0.01

SD = Standard deviation; CV = coefficient of variation; HNE = high N environment; LNE = low N environment.

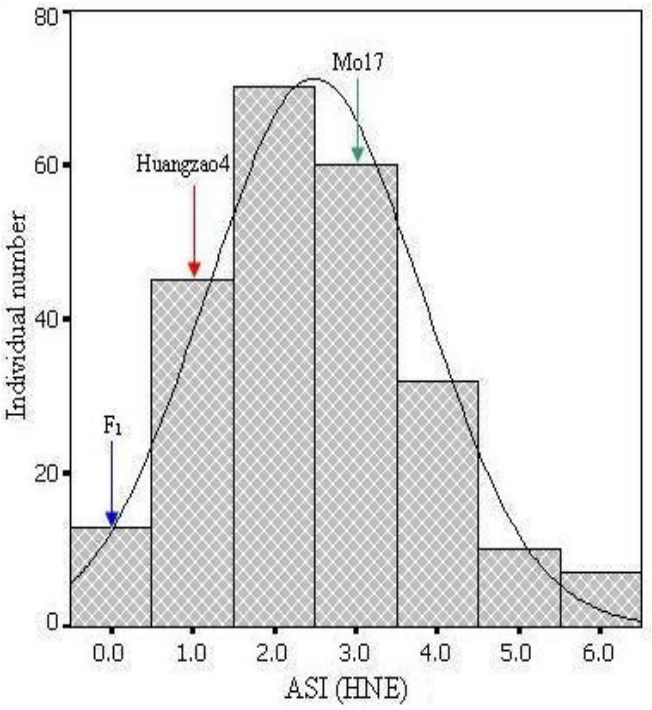

Figure 1. Frequency distribution of ASI in the RIL population consisting of 239 RILs under HNE

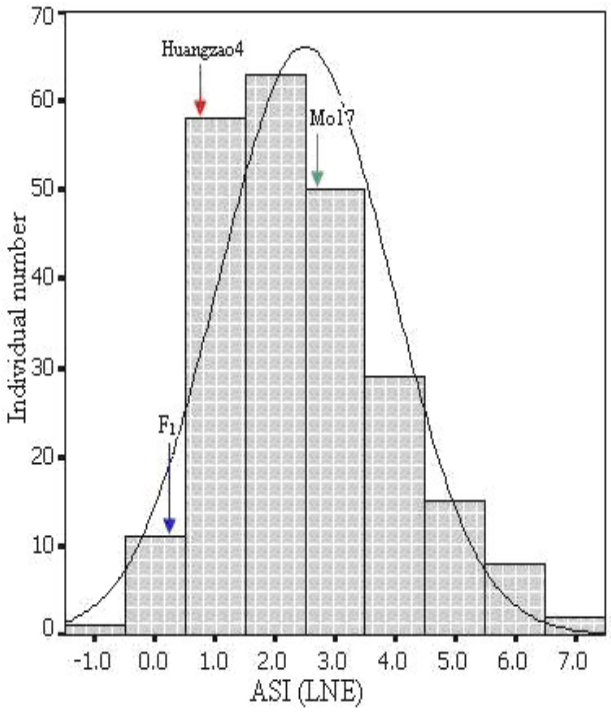

Figure 2. Frequency distribution of ASI in the RIL population consisting of 239 RILs under LNE.

Bnlg1863, respectively.

These QTLs identified could account for phenotypic variances from 4.08 to 17.10% and the total values were up to 53.67 and 31.87% under HNE and LNE, respectively (Table 2). The three QTLs including *Qasihn6-1*, *Qasihn8-2* and *Qasiln6-1* provided positive additive effects and could increase ASI, while, the others could decrease ASI due to negative additive effects.

DISCUSSION

N deficiency will severely affect many metabolic pathways and physiological progresses in maize (Ribaut et al. 2007), and increasing of ASI is one of the most serious consequences (Jacobs and Pearson, 1991). To realize the genetic basis of controlling ASI, in this study, the RIL population, derived from the cross between Mo17 and Huangzao4, was used to identify the QTLs controlling ASI under different N environments. As a result, 6 and 3 QTLs were detected under HNE and LNE, respectively. But, to be noticed, the two QTLs *Qasihn7-1* and *Qasiln7-1* were mapped on same chromosomal position, linked with marker Bnlg1792, which suggested that the two QTLs were probably same QTL, and could decrease ASI under the two N environments duo to negative additive effect. Similar result was also found in *Qasihn8-1* and *Qasiln8-1*.

Compared to the report by Ribaut et al. (2007), similar N environments were designed in QTL mapping for ASI, but the present studies showed a different report from theirs in several aspects and the main differences are displayed in Table 3. From the chromosomal position of linked markers, all the QTLs but *Qasihn8-2* (bin8.06) identified in the present experiments were obviously different from those reported by them. These results could probably be explained by the differences of parents, segregating population or marker type, similar to the two studies Xu et al. (1999) and Chen et al. (2005).

Regarding QTL mapping for ASI using Huangzao4 as short-ASI parent, besides this experiment, Li et al. (2003) reported that under different water regimes, 3 and 2

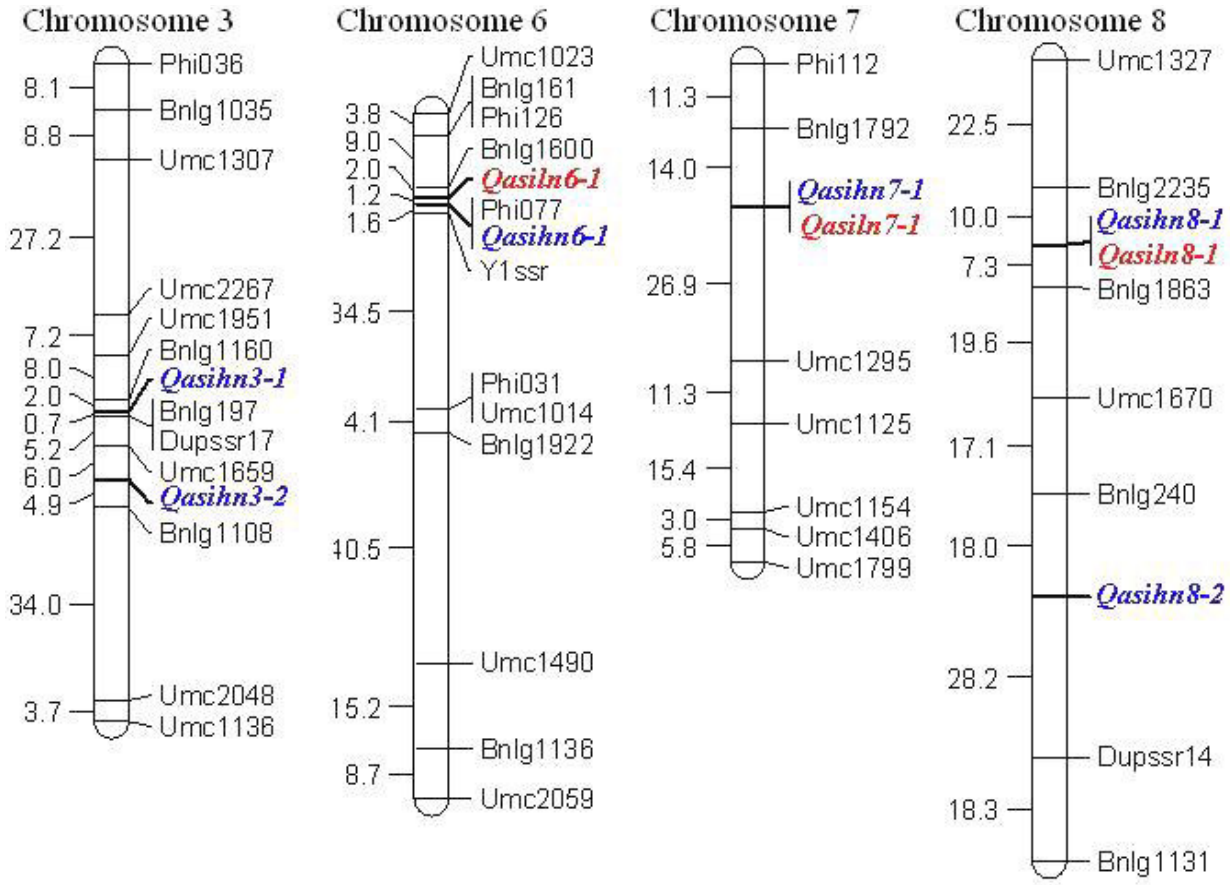

Figure 3. The position of QTLs conferring ASI identified under high and low N environments. The six including *Qasihn3-1, Qasihn3-2, Qasihn6-1, Qasihn7-1, Qasihn8-1 and Qasihn8-2* were detected under HNE (blue), while the three including *Qasiln6-1, Q asiln7-1 and Qasiln8-1* were detected under LNE (red).

Table 2. The QTLs controlling ASI mapped using RIL population from Mo17 × Huangzao4.

Environments	QTLs	Chr.	Flanking markers	Linked markers (interval)	LOD	R^2 (%)	Additive effects
HNE	*Qasihn3-1*	3	Bnlg1160 - Bnlg197	Bnlg197 (0.7 cM)	3.86	6.64	- 0.35
	Qasihn3-2	3	Umc1659 - Bnlg1108	Umc1659 (6.0 cM)	3.82	7.70	- 0.38
	Qasihn6-1	6	Phi077 - Y1ssr	Phi077 (0 cM)	2.56	4.08	0.27
	Qasihn7-1	7	Bnlg1792 - Umc1295	Bnlg1792 (14.0 cM)	2.60	12.27	- 0.48
	Qasihn8-1	8	Bnlg2235 - Bnlg1863	Bnlg1863 (7.3 cM)	3.36	8.28	- 0.40
	Qasihn8-2	8	Bnlg240 - Dupssr14	Bnlg240 (18.0 cM)	2.61	14.70	0.51
LNE	*Qasiln6-1*	6	Bnlg1600 - Phi077	Phi077 (1.2 cM)	3.80	6.53	0.37
	Qasiln7-1	7	Bnlg1792 - Umc1295	Bnlg1792 (14.0 cM)	3.74	17.10	- 0.60
	Qasiln8-1	8	Bnlg2235 - Bnlg1863	Bnlg1863 (7.3 cM)	3.23	8.24	- 0.42

LOD = Likelihood of odds; HNE = High N environment; LNE = low N environment.

QTLs were detected under well-watered and drought-stressed regimes, respectively. The main differences between the two studies are listed in Table 3. In addition, marker and individual number were also different, only 89 SSR markers and 184 individuals were used by Li et al. (2003), much less than the present experiments which made use of 100 SSR markers and 239 RILs. More saturated map and more individual would probably increase experimental reliability (Liu et al., 2009). Furthermore, to be mentioned, the five loci controlling ASI, including 3.06, 3.07, 6.01, 7.02, 8.03 and 8.06, were first detected in Huangzao4.

Table 3. QTLs identified for ASI under different environments.

Parents	Mapping populations	Marker type	Environments	Position of linked markers (bin)	References
Ac7643S5, Ac7729/TZSRWS5	$F_{2:3}$	RFLP	High N	1.08, 6.05, 7.05, 10.03	Ribaut et al. (2007)
			Low N	1.08, 3.05, 4.08, 6.05, 6.07, 7.04, 8.02, 8.06, 10.03	
Huangzao4, Ye107	$F_{2:3}$	SSR	Well water	1.01, 2.03, 3.01	Li et al. (2003)
			Drought stress	2.02, 5.05	
L050, B73	$F_{2:3}$	SSR	Well water	2.02, 6.07	Zhang et al. (2004)
			Drought stress	6.07	
B73, Tx303	$BC_3F_{2:3}$	RFLP, SSR	Well water	3.07, 9.05	Szalma et al. (2007)
			Drought stress	1.11, 9.02, 10.03	
$BC_3F_{2:3}$, Mo17	$BC_3F_{2:3}$×Mo17	RFLP, SSR	Well water	4.00, 4.04, 4.10, 5.07, 9.05	
			Drought stress	2.09, 3.06, 5.00, 5.04, 5.05, 5.05, 5.06, 6.01, 9.05, 9.07	
A188, 91huang15	$F_{2:3}$	SSR	Well water	2.03, 5.07, 6.01, 9.06-9.07	Wu et al. (2008)
			Drought stress	2.02, 4.05, 6.02	
Huangzao4, Mo17	RIL	SSR	High N	3.06, 3.07, 6.01, 7.02, 8.03, 8.06	This study
			Low N	6.01, 7.02, 8.03	

Except for above studies, some other short-ASI parents and environments were also used to analyze the QTL controlling ASI (Zhang et al., 2004; Szalma et al., 2007; Wu et al., 2008), but, the number and location of QTLs identified were different in different reports (Table 3). The variation of previous results was probably because of the differences of parents, segregation populations or molecular markers.

To be noticed, the previous mapping populations were focused on F_2 and BC (Li et al., 2003; Zhang et al., 2004; Szalma et al., 2007; Ribaut et al., 2007; Wu et al., 2008), the two kinds of segregating population were temporary and could not be reused in QTL mapping, for they could not supply continued plants for phenotypic and genetic analysis (Pilet et al., 2001). However, RIL population applied in this experiment was immortal and could be used again and again in different regions and time due to homogenous individuals. Thus, some markers could be added into given chromosomal regions and the QTLs controlling ASI analyze once more. This is similar to previous reports (You et al., 2006; Balint-Kurti and Carson, 2006).

Furthermore, the locus *Qasihn6-1* identified in the present studies was quite near to marker phi077, with 0 cM of genetic distance. This suggested that the linked marker phi077 could probably be co-segregated with the gene controlling ASI within *Qasihn6-1*, and thus, could be used for MAS in maize breeding program. In addition, the QTL *Qasihn3-1* was very close to marker bnlg197, with only 0.7 cM of genetic interval and it could decrease ASI due to negative additive effect. Some markers could be added into the chromosomal region, to shorten the genetic distance between *Qasihn3-1* and bnlg197. Other QTLs identified were far to their linked markers except for *Qasiln6-1* with only 1.2 cM of genetic interval. At present, the research work for fine mapping the QTLs controlling ASI is in progress based on the constructed RIL population and genetic map.

In summary, an RIL population consisting of 239 RILs, derived from the cross between Mo17 and Huangzao4, was used to identify the QTLs controlling ASI under different N environments. As a result, 6 QTLs were detected under HNE on chromosome 3, 6, 7 and 8 and could explain 53.67% of phenotypic variance. Under LNE, 3 QTLs were mapped on chromosome 6, 7 and 8 and could account for 31.87% of phenotypic variance. The two QTLs including *Qasihn6-1* and *Qasihn3-1* identified under HNE were quite near to their linked marker, with less than 1 cM of genetic distance and could be considered as candidate loci in MAS. These results are beneficial for understanding the genetic basis of ASI in maize.

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