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Full Length Research Paper

A quantitative trait locus for the number of days from sowing to seedling emergence in maize

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Quantitative trait locus (QTL) mapping provides useful information for breeding programs since it allows the estimation of genomic locations and genetic effects of chromosomal regions related to the expression of quantitative traits. The number of days from sowing to seedling emergence (NDSSE) is an important agronomic trait in a maize (*Zea mays* L.) breeding project which is related to yield. To determine its genetic basis, a recombinant inbred line (RIL) population and two nitrogen (N) regimes were used to detect the QTLs associated with NDSSE; as a result, one QTL was identified under high N regime, on chromosome 9, which could explain 6.20% of phenotypic variance and a decrease of 0.18 of NDSSE due to an additive effect. These results are beneficial for understanding the genetic basis of NDSSE in maize breeding project.

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

INTRODUCTION

The number of days from sowing to seedling emergence (NDSSE) is one of the most important agronomic traits, which is related to yield in maize (*Zea mays* L.). Too high NDSSE will postpone harvest, while excessively low NDSSE will result to harvest before harvest time. Quantitative trait locus (QTL) mapping is an effective method to understand genetic basis of a trait. To date, many important traits in maize have been studied at the molecular level (Ribaut et al., 2007; Liu et al., 2008, 2009). However, limited QTL mapping studies have focused on NDSSE in maize.

Both parental lines and segregating population have some effects on QTL mapping results to some extent. At present, many elite maize inbred lines have been used in QTL mapping, and large numbers of QTLs associated with agronomic traits have been located on maize chromosomes (Trachsel et al., 2009; Ruta et al., 2010). However, previous mapping popu-lations were focused on F_2, whereas RIL population, possessing the merit immortality, has less application in QTL detection for maize growth period including NDSSE. Moreover, ecological condition is another important factor affecting QTL identification, because genes might be expressed differently in different environments. For example, using F_2 population from the cross X178 × B73, Xiao et al. (2005) identified two QTLs on chromosomes 1 and 9 under well-water environment, while under water-stressed environment, three QTLs were mapped on chromosomes 1, 2 and 9. To date, most studies on QTL mapping were focused on water stress in the soil, involving different water regimes in the same field, or different experimental regions possessing different rainfall rate (Guo et al., 2008; Messmer et al., 2009), whereas, hardly any QTL for NDSSE studies have been done on N efficiency in maize.

Therefore, in this present study, an F_9 RIL population derived from the two maize parental lines Mo17 and Huangzao4 were used to identify the QTLs associated with NDSSE under different N regimes, the objectives of

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Abbreviations: QTL, Quantitative trait locus; **NDSSE,** number of days from sowing to seedling emergence; **RIL,** recombinant inbred line.

Table 1. The statistical values of the three lines including Mo17, Huangzao4 and F_1 on NDSSE.

N regime	Mean of NDSSE (d)		
	M017	Huangzao4	F_1
High N	11.00	9.67	9.33
Low N	11.00	9.33	9.00

Table 2. The descriptive statistics of the RIL population under HNR and LNR on NDSSE.

N regime	Minimum (d)	Maximum (d)	Mean (d)	SD[a]	Skewness	Kurtosis
High N	8.33	13.00	9.83	0.73	0.56	0.81
Low N	8.00	12.67	9.44	0.65	0.94	2.58

[a] Standard deviation.

this study were to identify the chromosomal loci associated with the trait NDSSE, and to further understand its genetic basis.

MATERIALS AND METHODS

Plant materials

The materials involved in this study included parental inbred lines Mo17 (high NDSSE) and Huangzao4 (low NDSSE), F_1 and an F_9 segregating population consisting of 239 RILs. Mo17 and Huangzao4 are the representative lines of American Lancaster and Chinese Tangsipingtou heterotic groups, respectively. F_1 and RIL population were derived from the cross between Mo17 and Huangzao4. Comparatively, under the same N environment, Mo17 has high NDSSE than Huanzao4; certainly, they might present difference in NDSSE under different N environments.

Field experimental designs and measurements

All the 242 lines were sown in a complete randomized design with six replicates at Nanchong Institute of Agricultural Sciences, Nanchong City, People's Republic of China, with one plant per hill and 15 plants per row as one replicate. Among the six replicates for one line, three were under high N regime (HNR) by appending urea 300 kg/ha and the other three 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.

Data analysis

At the time of seedling emergence, the middle eight plants of every replicate of each line were individually investigated on the trait NDSSE, based on the investigated data of the RIL population, and SPSS11.5 software (www.spss.com) was used to perform descriptive statistics, analysis of variance (ANOVA) and correlation analysis.

QTL mapping

Based on the phenotypic data of the RIL population under two N regimes and the genetic map consisting of 100 simple sequence repeat markers and covering 1421.5 cM of mapping distance (Liu et al., 2009), the QTLs associated with NDSSE were identified by composite interval mapping (CIM) of Windows QTL Cartographer 2.5 software (Wang et al., 2010). Control parameters included standard CIM model, 2.0 cM as walk speed, 5 control markers, 10-cM window size and forward regression method. The threshold value for the QTL significance was determined by 1000-time permutation test ($\alpha = 0.05$) (Doerge and Churchill, 1996). The QTL(s) with a LOD ($\log_{10}$ of odds ratio) value greater than the threshold value was presented, and their position, percentage of phenotypic variation and genetic effects were estimated at the significant LOD peak in the region, then, the identified QTLs were mapped with Mapchart 2.1 software (Voorrips, 2002).

RESULTS

Phenotypic observation and statistical analyses

The results of investigation on NDSSE showed that the tested lines presented variations. For the three lines, Mo17, Huangzao4 and F_1, under both N regimes, Mo17 had the highest value, followed by Huangzao4; F_1 had the lowest NDSSE (Table 1). For the RIL population, the results of ANOVA demonstrated that the 239 RILs under both N regimes provided differences at 0.01 probability level, nevertheless, the two group data obtained under two N regimes presented significant positive correlation at 0.01 probability level, with a correlation coefficient value of 0.789.

The results of the descriptive statistics for the RIL population are displayed in Table 2, among the six statistical parameters, these four values including minimum, maximum, mean and standard deviation (SD) under HNR were higher than those under LNR, while, skewness and kurtosis had contrary statistic results.

QTL identification

The results of permutation test showed that the QTL

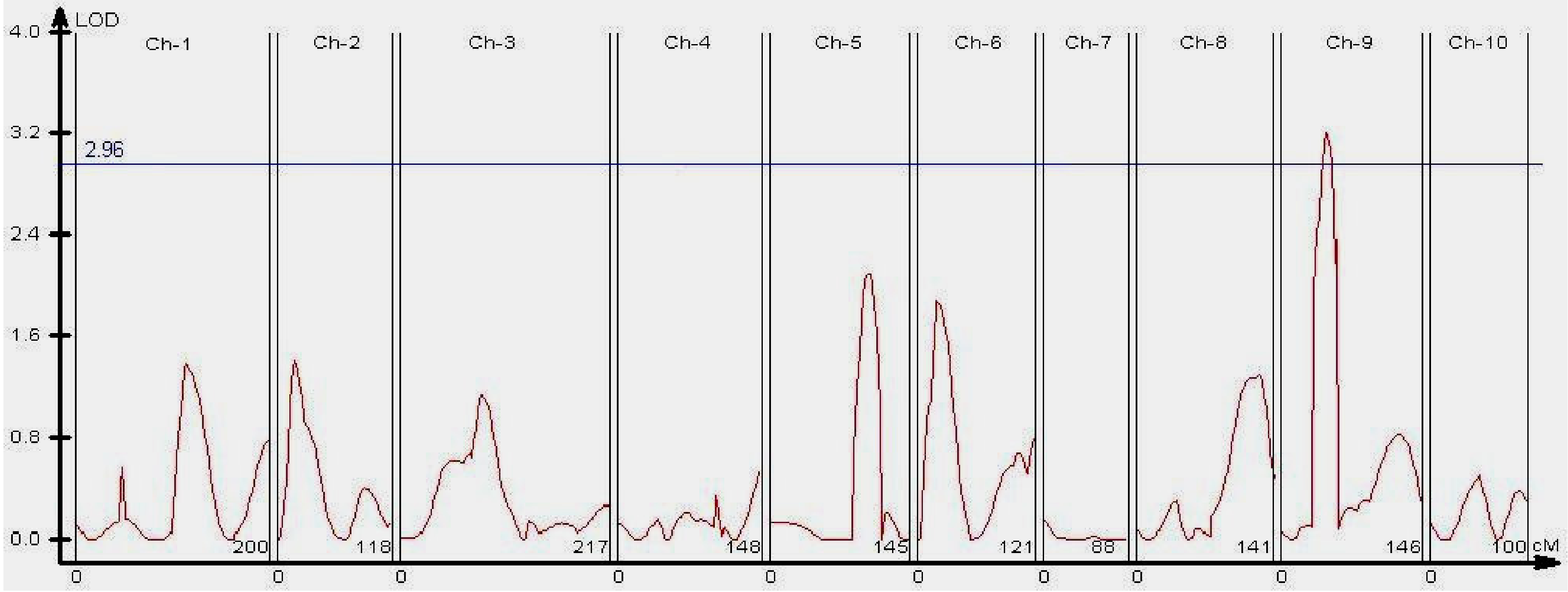

Figure 1. QTL scanning for NDSSE under HNR based on the RIL population from the cross Mo17 × Huangzao4. One QTL was detected on chromosome 9, in a condition where LOD threshold was set at 2.96.

significance threshold should be set 2.96 and 2.70 for the RIL population under HNR and LNR, respectively. Based on the premises, under HNR, one QTL was mapped on chromosome 9 (Figure 1), while under LNR, no any QTL was detected. The QTL identified under HNR was flanked by Umc1893 and Phi022 (Figure 2), with 6.0 and 2.1 cM of mapping interval, respectively, and could explain 6.20% of phenotypic variance and a decrease of 0.18 d owing to additive effect (Table 3). According to the phenotypic data of parental lines and additive effect of the QTL, it was concluded that the QTL on chromosome 9 were from Huangzao4.

DISCUSSION

Most of the agronomic traits in maize are quantitative. Because of the environmental effects, difficulties arise to correctly determine the genotype of individuals. In order to work around this problem, sophisticated quantitative methods have been developed, including QTL mapping, by means of statistical associations between markers and regions along the chromosomes, seeking to provide a better understanding on the genetic architecture of quantitative traits (Sabadin et al., 2008).

QTL mapping depends on a segregation population, at present; many types of the population have been successfully applied in quantitative loci identification of traits of interest (Agrama et al., 1999; Bovill et al., 2010; Fu et al., 2010). From literature, previous studies focused on F_2 segregating population (Khairallah et al., 1998; Szalma et al., 2007), because they have less time and labor intensive to develop, however, F_2 population has a limitation because it can be utilized only once, whereas, RIL population is stable and can be used again and again in different regions and time, though they require higher investment in time and manpower to develop. Currently, RIL populations are being widely used

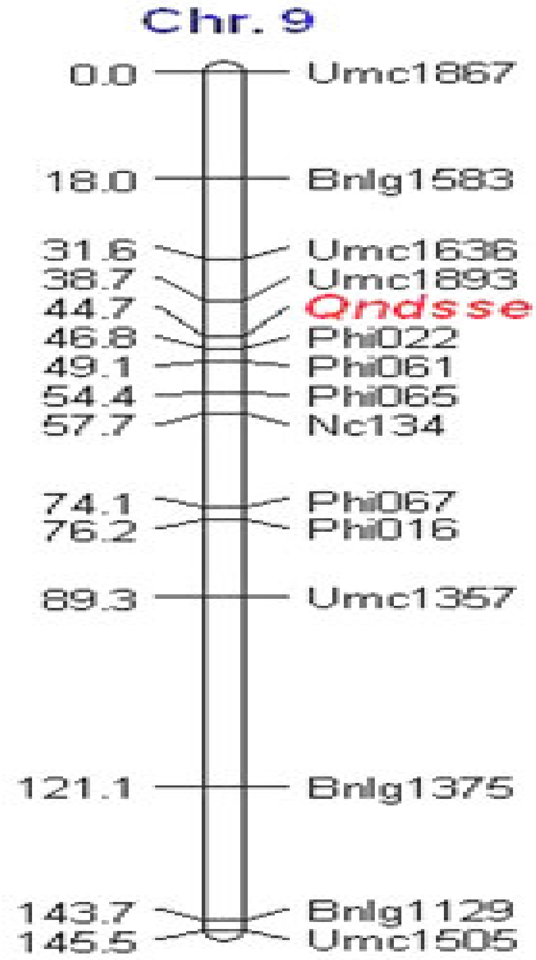

Figure 2. The chromosomal position of the QTL for NDSSE identified under HNR based on the RIL population from the cross Mo17 × Huangzao4. One QTL named *Qndsse* was located on chromosome 9, flanked by Umc1893 and Phi022.

Table 3. The QTL for NDSSE identified under HNR based on the RIL population from Mo17 × Huangzao4.

QTL name	Chromosome	Flanking marker	Position (cM)	LOD[a]	R^2(%)[b]	Additive effect
Qndsse	9	Umc1893-Phi022	44.71	3.22	6.20	-0.18

[a]Log_{10} of odds ratio; [b]Percentage of phenotypic variance explained by QTL.

in QTL mapping for agronomic traits in crops owing to their stability (Wan et al., 2006; Yang et al., 2010). Moreover, ecological conditions can affect the results of QTL location, and thus, it must be considered in experimental design. N is one of the most important chemical elements for maize growth and development (Liu et al., 2010), for the same gene, it can present differential expression under different N conditions in soil. For example, using a segregating population consisting of 240 $F_{2:3}$ families derived from two tropical lines to detect the QTLs associated with chlorophyll content in ear leaf, Ribaut et al. (2007) identified four QTLs under low N regime on chromosomes 1 (one), 4 (two) and 7 (one); while under high N regime, total five QTLs were located on chromosomes 1, 4, 6, 9 and 10.

NDSSE is a very important agronomic trait in maize breeding program, related to seedling emergence and quality. However, its genetic basis has hardly been studied to this day. So in this study, to realize its genetic base, a RIL population from the cross between Mo17 (high NDSSE) and Huangzao4 (low NDSSE) and two N regimes were used to detect the QTL for NDSSE. As a result, one QTL was mapped on chromosome 9 under HNR, flanked by Umc1893 (bin 9.02) and Phi022 (bin 9.03). Because the trait of maize has not been studied on QTL mapping from previous literature, this result can not be compared to other studies. Although, the mapping distances between the identified QTL and its flanking markers are far, some other molecular markers could be added to this given chromosomal regions to map the QTL more finely. Currently, further work is in progress based on the RIL population and genetic map established by us in previous experiments.

In summary, the RIL population consisting of 239 RILs from parental lines Mo17 and Huangzao4 and two N regimes were used to detect the QTLs associated with NDSSE in maize, the results showed that one QTL was identified under HNR on chromosome 9, flanked by Umc1893 and Phi022, with 6.0 and 2.1 cM of mapping interval, respectively. The QTL from Huangzao4 could account for 6.20% of phenotypic variance and decrease 0.18 of NDSSE due to additive effect. These results are beneficial for understanding the genetic basis of NDSSE in maize breeding projects.

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