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Full Length Research Paper

Identification of the QTLs for grain yield using RIL population under different nitrogen regimes in maize

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Quantitative trait locus (QTL) mapping can provide 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. To realize the genetic basis of grain yield of maize (*Zea mays* L.), a recombinant inbred line (RIL) population and two nitrogen (N) regimes were used to detect the QTLs for grain yield in maize, as a result, a total of six QTLs associated with grain yield per year (GYPE) were identified on chromosomes 1 (one), 6 (one), 8 (two) and 9 (two), with 0 - 12.0 cm of mapping interval between QTLs and their nearest markers. The three QTLs identified under high N regime could explain 18.07% of phenotypic variance, and could increase GYPE from 3.91 - 5.40 g, due to positive additive effects. Whereas, the three QTLs located under low N regime could account for 20.96% of phenotypic variance, and due to negative additive effects, they could decrease GYPE from 3.40 to 6.68 g. These results were beneficial for realizing the genetic basis of GYPE and developing marker-assisted selection in maize breeding project.

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

INTRODUCTION

Maize (*Zea mays* L.) is among crops of greatest economic importance in the world, and has been used as human and animal food, as well as raw material in the high-technology industry. As for breeding, it is one of the most studied species and has been applied as a model in many situations. Among the various traits normally considered in breeding research, grain yield is generally the most important one (Sabadin et al., 2008). But at present, available resources are very limited and conventional breeding method is laborious and time-consuming (Ribaut et al., 1997); this reduced the progress of yield breeding to some extent. To resolve this problem, an effective approach is to utilize high-yield genes in maize to improve the trait, but, this must depend on understanding of genetic basis.

Quantitative trait locus (QTL) mapping is an effective method to realize genetic basis of agronomic traits. At present, many QTLs associated with grain yield have been identified (Coque et al., 2006; Ribaut et al., 2007; Sabadin et al., 2008), but different parental lines, segregation population or genetic map can bring about different results in QTL number, position or effect, and thus, it is necessary and significant that different parents be selected to detect the QTLs for grain yield (Christov et al., 2004; Tomlekova et al., 2010). Moreover, previous populations were focused on F_2 (Ribaut et al., 2007; Sabadin et al., 2008), this kind of segregation population has a deficiency named temporality, because no continued plant is used for phenotypic analysis and DNA extraction.

Relatively, recombinant inbred line (RIL) population is immortal, and can be used again and again in different regions and time due to humongous individuals. At present, RIL population has been widely used to dissect the QTLs controlling agronomic traits in crop (Wan et al., 2006; McIntyre et al., 2010; Yang et al., 2010), nevertheless, only few studies on QTL mapping for maize

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Table 1. Phenotypic values of parental lines and F_1 in GYPE.

N regimes	Mo17	Huangzao4	F_1
High N	41.43	59.23	178.81
Low N	52.59	48.86	163.11

grain yield using RIL population were reported in literatures to date (Coque et al., 2006; Ma et al., 2007; Messmer et al., 2009; Tang et al., 2010).

Furthermore, ecological conditions can affect gene expression, and same gene will probably present expression variation under different environments. For example, using F_2 population derived from the cross X178 × B73, Xiao et al. (2005) identified six QTLs for cob weight per ear under water-stressed regime, each one on chromosomes 1, 3, 5 and 7 and two on chromosome 9, while under well-watered regime, only one QTL was detected, on chromosome 1. According to published literature, previous environments designed for QTL identification for maize grain yield were centered on different water-content conditions (Frova et al., 1999; Lu et al., 2006; Guo et al., 2008; Messmer et al., 2009), whereas, different nitrogen (N) content in soil is hardly used for QTL analysis, although it is one of the most important influencing factor on agronomic traits in maize, especial for grain yield.

Therefore, in this present study, an F_9 RIL population from the cross Mo17 × Huangzao4 and two N regimes were used to detect the QTLs associated with grain yield, the objectives were to (1) identify and evaluate the QTLs for grain yield, and (2) further realize the genetic basis of grain yield in maize.

MATERIALS AND METHODS

Plant materials

The experimental materials involved in this study included maize inbred lines Mo17 and Huangzao4, F_1 and an F_9 RIL population consisting of 239 RILs. Mo17 and Huangzao4 are the representative lines of Lancaster and Tangsipingtou heterotic groups, respectively, the F_1 and RIL population were derived from the cross between the two parental lines Mo17 and Huangzao4.

Field experiments and statistics analyses

All the 242 lines were sown in a complete randomized design with six replicates at the experimental field of Nanchong Institute of Agricultural Sciences, Nanchong City, People's Republic of China, with single-plant planting, 15 plants per row and one ear per plant as a replicate, of which three replicates 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.

During harvest, the eight middle plants of every replicate of each line were individually investigated and their mean was computed on the trait grain yield per ear (GYPE), based on means from 24 plants of each line under same N regime, SPSS11.5 software (www.spss.com) was performed on descriptive statistics, analysis of variance (ANOVA) and correlation analysis.

QTL mapping

Based on the means of each line of the population obtained under HNR and LNR respectively, and the genetic map consisting of 100 simple sequence repeat (SSR) markers and covering 1421.5 cm of mapping distance (Liu et al., 2009), the QTLs associated with GYPE were identified by composite interval mapping (CIM) of Windows QTL Cartographer 2.5 software (Wang et al., 2010), 2.0 cm as walk speed and $\log_{10}$ of odds ratio (LOD) 2.0 as QTL significance threshold as described in previous reports (Fontaine et al., 2003; Okogbenin et al., 2006; Wang et al., 2007). Control parameters included standard CIM model, 5 control markers, 10-cm window size and forward regression method. The QTLs with an 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. Then, the identified QTLs were mapped with Mapchart 2.1 software (Voorrips 2002).

RESULTS

Phenotypic observation and statistic analysis

The results investigated on GYPE showed that the tested lines presented variations. With respect to the three lines including Mo17, Huangzao4 and F_1, F_1 had much higher GYPE than parental lines under both N regimes (Table 1), this could be explained by heterosis. Under HNR, the GYPE of Huangzao4 was higher than that of Mo17, while under LNR, the result was contrary. For the RIL population, the valid 236 RILs (excluding three missing data) under both HNR and LNR provided significant differences in GYPE at 0.01 levels (Table 2). Nevertheless, the two group data presented significant positive correlation at 0.01 levels, with 0.878 of correlation coefficient.

The results of the descriptive statistics for GYPE were displayed in Table 3. Among the eight statistics parameters, except for standard deviation, coefficient of variation and skewness, all of them presented higher values under LNR than those under HNR. In addition, from the frequency distribution graphs of the RIL population under two N regimes (Figures 1 and 2), the two group data could well agree with normal distribution, which suggested that GYPE was a quantitative trait and controlled by multiple genes.

QTL identification

Mapping software was used to detect the QTLs for GYPE, as a result, three QTLs were detected under HNR, one on chromosome 8 (*Qy-hn-1*) and two on chromosome 9 (*Qy-hn-2* and *Qy-hn-3*), near to Bnlg1893,

Table 2. ANOVA of the RIL population on GYPE under two N regimes.

N regimes	Variation sources	Sum of squares	[a]df	Mean square	*F*	Sig.
High N	Between groups	267580.93	235	1138.64	8.31**	<0.01
	Within groups	64669.89	472	137.01		
Low N	Between groups	233097.80	235	991.91	9.06**	<0.01
	Within groups	51660.72	472	109.45		

[a] There were three missing values among the RIL population consisting of 239 RILs. ** Significant difference at 0.01 level.

Table 3. Descriptive statistics of RIL population on GYPE under two N regimes.

N regimes	Range	Minimum	Maximum	Mean	[a]SD	[b]CV (%)	Skewness	Kurtosis
High N	114.18	5.33	119.51	52.85	19.48	36.86	0.056	0.177
Low N	118.95	11.42	130.37	56.69	18.18	32.08	0.054	0.769

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

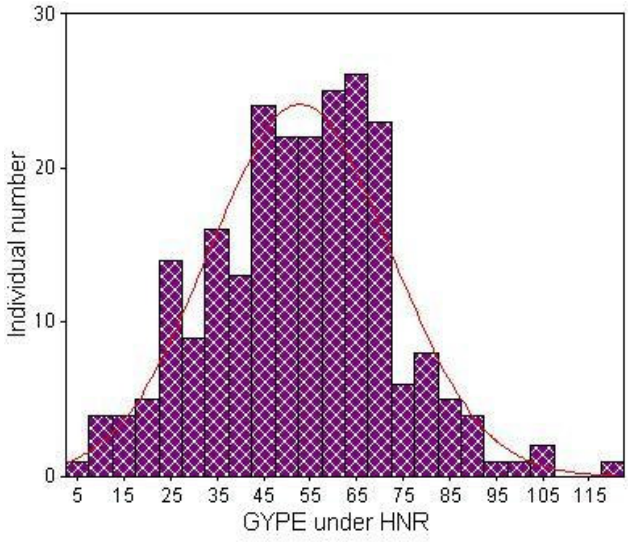

Figure 1. Frequency distribution graph of the RIL population for GYPE under HNR.

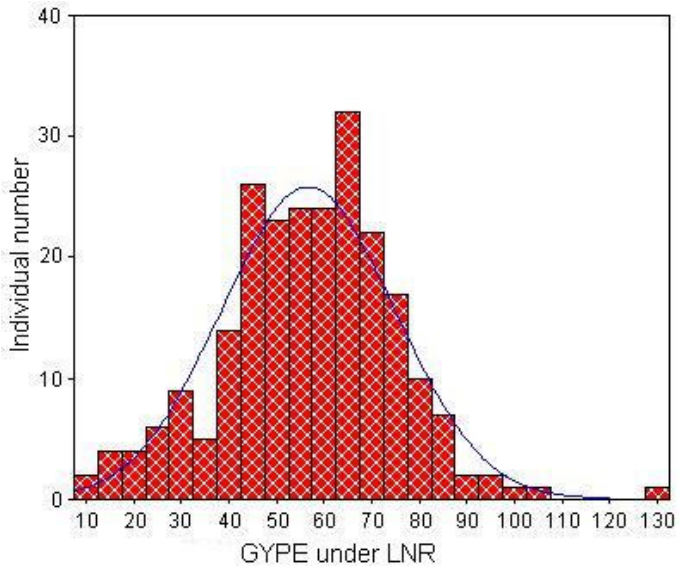

Figure 2. Frequency distribution graph of the RIL population for GYPE under LNR.

Phi061 and Nc134, respectively (Figure 3), the intervals between the QTLs and their nearest markers ranged from 0 to 6.0 cm. The three QTLs could explain a total 18.07% of phenotypic variance, and due to positive additive effects, could increase GYPE from 3.91 to 5.40 g (Table 4).

Under LNR, three QTLs were detected on chromosomes 1 (*Qy-ln-1*), 6 (*Qy-ln-2*) and 8 (*Qy-ln-3*), near to Phi308707, Bnlg1600 and Bnlg240, respectively (Figure 3), the mapping interval ranged from 0 to 12.0 cm. The three QTLs could account for total 20.96% of phenotypic variance, due to negative additive effects; they could decrease GYPE from 3.40 to 6.68 g (Table 4).

DISCUSSION

Grain yield in maize is a quantitative trait controlled by polygene (Lu et al., 2006; Guo et al., 2008). To realize its genetic basis, in this present study, a RIL population and two N regimes were used to detect the QTLs for GYPE, as a result, a total of six QTLs were identified, of which three were located under HNR, one on chromosome 8 and two on chromosome 9, while, the other three were detected under LNR, each one on chromosomes 1, 6 and 8. Although many studies on QTL identification for grain yield were reported in maize (Frova et al., 1999; Lu et al.,

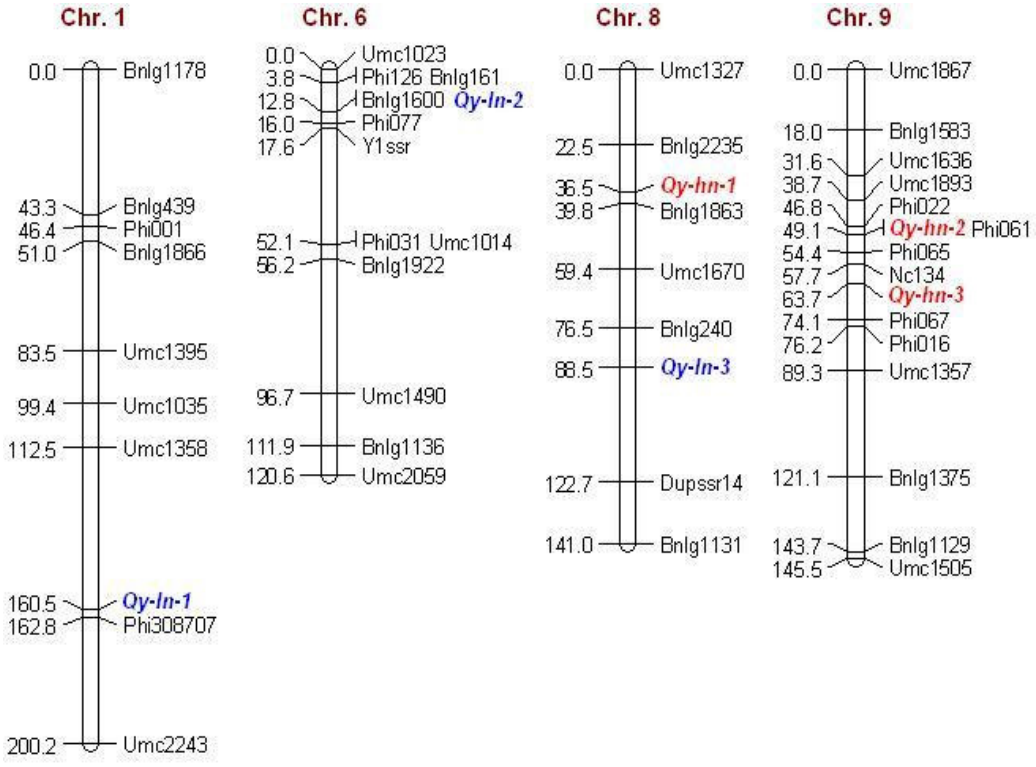

Figure 3. The chromosomal positions of QTLs for GYPE identified using RIL population under two N regimes. The three QTLs including *Qy-hn-1*, *Qy-hn-2* and *Qy-hn-3* were detected under HNR (red), while the three QTLs including *Qy-ln-1*, *Qy-ln-2* and *Qy-ln-3* were detected under LNR (blue).

Table 4. The QTLs for GYPE identified using RIL population under two N regimes.

N regimes	QTL name	No. of chromosomes	Nearest marker	Position (cm)	Interval (cm)	[a]LOD	[b]R^2 (%)	Additive effect
High N	*Qy-hn-1*	8	Bnlg1863	36.5	3.3	2.94	6.67	5.23
	Qy-hn-2	9	Phi061	49.1	0	2.03	3.81	3.91
	Qy-hn-3	9	Nc134	63.71	6.0	2.43	7.59	5.40
	Total						18.07	
Low N	*Qy-ln-1*	1	Phi308707	160.5	2.3	2.17	4.56	-3.99
	Qy-ln-2	6	Bnlg1600	12.8	0	2.05	3.46	-3.40
	Qy-ln-3	8	Bnlg240	88.51	12.0	2.42	12.94	-6.68
	Total						20.96	

[a]LOD, $\log_{10}$ of odds ratio. [b]R^2, percentage of phenotypic variance explained by QTL.

2006; Ribaut et al., 2007; Sabadin et al., 2008; Guo et al., 2008), it is not easy to compare previous results with ours, because these differences, including environmental design, genetic maps, markers used and statistical methods, will make comparison difficulties. Therefore, the comparisons here were focused on the selections of segregation population and ecological conditions.

Previous population, used for QTL mapping for grain yield, was focused on F_2 (Ribaut et al., 1997, 2007; Sabadin et al., 2008). This kind of population is temporary and can not be reused, because there are no continued plans used for phenotypic analysis and DNA extraction. Relatively, RIL population, employed in our study, is immortal and can be applied again and again, due to homologous individuals. At present, RIL population has widely been employed in QTL mapping in crop, including rice (*Oryza sativa* L.) (Lu et al., 2006; Gao et al., 2007), wheat (*Triticum aestivum* L.) (Zhang et al., 2008) and maize (Messmer et al., 2009), but till date, only few studies on QTL studies for maize grain yield were

Table 5. The QTLs for yield identified using RIL population in maize.

References	Parental lines	Chromosomal name (QTL number)
Coque et al. (2006)	F2, Io, F252 as tester,	1 (one), 2 (four), 4 (two), 5 (three), 6 (one)
Tang et al. (2010)	Zong3, 871	1 (two), 8 (one)
Messmer et al. (2009)	CML444, SC-Malawi	1 (one), 5 (three), 8 (one)
Ma et al. (2007)	Z3, 871	1 (two), 4 (one), 5 (one), 6 (one), 7 (two)
This study	Mo17, Huangzao4	1 (one), 6 (one), 8 (two), 9 (two)

found in literature (Coque et al., 2006; Ma et al., 2007; Messmer et al., 2009; Tang et al., 2010). Compared to previous studies, our results were different from the previous in many aspects, despite similar RIL population, and the main differences were listed in Table 5. Moreover, it is worthy of note that, some QTLs reported by different researches were located on same chromosomes, but their chromosomal bin loci were different from each other, for example, the QTL on chromosome 6 identified by Ma et al. (2007) was within bin 6.05, while the QTLs on the same chromosome reported by Coque et al. (2006) and us were within bin 6.1 and 6.0, respectively. These differences could be explained by different parental lines, genetic map or ecological conditions.

Previous ecological conditions, designed for QTL identification for grain yield of maize, were mainly different water content in soil (Frova et al., 1999; Lu et al., 2006; Guo et al., 2008), whereas, different N regimes were hardly used (Agrama et al., 1999; Liu et al., 2007), although N fertilizer is an important factor affecting grain growth and development. Compared to previous reports, our results still differed greatly from the previous, including QTL number, position and genetic effects, which could result from different parental lines, segregation population or genetic map.

Furthermore, in our study, the two QTLs *Qy-hn-2* and *Qy-ln-2* were quite near to their linked markers, with only 0 cm apart, which suggested that the linked makers could probably be co-segregated with the loci controlling GYPE, and thus, could be considered for marker-assisted selection (MAS) in maize breeding project. While, the other four QTLs were far away from their linked markers, with a range from 2.3 to 12.0 cm apart, for this, other molecular markers should be added to the given regions for mapping these QTLs more finely, further work is in progress based on the RIL population and genetic map.

In summary, a RIL population and two N regimes were used to detect the QTLs for grain yield in maize, as a result, a total of six QTLs were identified, on chromosomes 1 (one), 6 (one), 8 (two) and 9 (two), with 0 - 12.0 cm apart between QTLs and their nearest markers. The three QTLs identified under HNR could explain 18.07% of phenotypic variance, and could increase GYPE from 3.91 to 5.40 g, owing to positive additive effects. Whereas, the three QTLs, mapped under LNR, could account for 20.96% of phenotypic variance, due to negative additive effects, they could decrease GYPE from 3.40 to 6.68 g. These results were beneficial for realizing the genetic basis of GYPE and developing MAS in maize breeding program.

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