African Journal of Biotechnology Vol. 9(47), pp. 8007-8012, 22 November, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1062
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Identification of the quantitative trait loci for grain rate in maize

Xiaohong Liu[1], Sulan He[2], Zuping Zheng[2]*, Zhenbo Tan[3] and Daihui Liu[2]

[1]College of Life Sciences, China West Normal University, Nanchong City 637009, People's Republic of China; Key Laboratory of Southwest China Wildlife Resources Conservation (Ministry of Education), People's Republic of China.
[2]Nanchong Institute of Agricultural Sciences, Nanchong City 637000, People's Republic of China.
[3]Beijing IPE Bio-technology Co., Ltd, Beijing City 100085, People's Republic of China.

Accepted 23 September, 2010

Grain rate (GR) is a very important trait in maize (*Zea mays* L.) breeding program related to yield. To realize its genetic basis, a recombinant inbred line (RIL) population and different nitrogen (N) regimes were used to map the quantitative trait loci (QTLs) for GR in maize. As a result, two QTLs were identified under high N regime and could explain a total of 14.84% of phenotypic variance. Due to additive effect, the QTL on chromosome 6 could decrease 0.029 of GR, while the QTL on chromosome 9 could increase 0.0203 of GR. Under low N regime, one QTL was mapped on chromosome 6 and could account for 9.52% of phenotypic variance, and owning to additive effect, the QTL could make GR decrease by 0.0234. The result in comparison with previous studies showed that the three QTLs in this present study were new quantitative loci associated with GR in maize. These results were beneficial for understanding the genetic basis of GR in maize.

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

INTRODUCTION

Grain rate (GR) is one of the most important traits in maize (*Zea mays* L.) breeding program (Yang et al., 2005) related to yield, but at present, the maize germplasm resources with high GR are quite lacking. To resolve this problem, an effective solution is to utilize elite genes associated with high GR to improve the trait of maize, but this must depend on an understanding on its genetic basis and quantitative trait locus QTL) mapping is an efficient approach to realize genetic basis of agronomic trait (Liu et al., 2009).

To date, there are many reports on QTL mapping for yield-related traits in maize, such as grain yield (Ribaut et al., 1997), ear weight (Sabadin et al., 2008), field grain drying rate (Sala et al., 2006), 100-kernel weight (Guo et al., 2008), grain row number per ear and grain number per row (Xiang et al., 2001), but for the trait GR, only few studies were found in literature (Yang et al., 2005; Li et al., 2009). Furthermore, the previous populations for QTL mapping affecting GR were focused on F_2. This kind of population is temporary and could not be reused, because there were no continued plants used for phenotypic and genetic analysis. However, recombinant inbred line (RIL) population is immortal and can be used again and again in different regions and time, due to homogenous individuals. 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 till date, the studies on QTL mapping associated with GR using this kind of population were hardly reported in maize.

Although some QTLs associated with GR have been mapped, different parental lines, segregation populations or ecological conditions will lead to different results including QTL number, location or effect. For example, using 48-2 and 5003 as parental lines, Yang et al., (2005)

*Corresponding author. E-mail: zzp0817@163.com. Tel: +86-13088139971.

Abbreviations: GR, Grain rate; **QTL,** quantitative trait locus; **RIL,** recombinant inbred line; **N,** nitrogen; **HNR,** high nitrogen regime; **LNR,** low nitrogen regime; **CIM,** composite interval mapping; **LOD,** $\log_{10}$ of odds ratio.

Table 1. Investigated values of parental lines and F_1 on GR.

N regime	Mo17	Huangzao4	F_1
High N	0.801	0.832	0.854
Low N	0.866	0.836	0.858

identified five QTLs for GR on chromosomes 1, 2, 5 and 8. Whereas in the report by Li et al. (2009), Qi319 and Huangzao4 were applied as mapping parents, as a result, only two QTLs were detected on chromosomes 3 and 5. And thus, to develop new loci for GR in maize, it was necessary and significant to select new elite parental lines to map the QTLs associated with GR.

Nitrogen (N) content in soil can affect gene expression; maybe same gene presents expression variation under different N supplies (Ribaut et al., 2007; Liu et al., 2007). Thus, different N regimes have frequently been used to detect QTLs 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). But till date, the studies on QTL mapping for GR using different N regimes were scarcely ever reported.

Therefore, in this present study, a RIL population descendant from the cross between Mo17 and Huangzao4 were used to map the QTLs for GR under two N regimes in maize. The objectives are to identify the QTLs associated with GR from new maize germplasm and to realize more clearly the genetic basis of GR in maize.

MATERIALS AND METHODS

Plant materials

The experimental materials used in this study included two elite maize inbred lines Mo17 and Huangzao4 as parents, 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; F_1 and the RIL population were derived from the cross between the two parental lines.

Field experiments

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, one ear per plant 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.

During harvest, the middle eight plants of each replicate were individually investigated on the GR trait according to the formula:

GR = weight of dry grain / weight of dry ear

Based on the investigated data of the RIL population, SPSS11.5 software (www.spss.com) was performed on analysis of variance (ANOVA), correlation analysis and descriptive statistics for the trait GR.

QTL mapping

Based on the phenotypic data of the RIL population under two N regimes and the genetic map consisting of 100 SSR markers and covering 1421.5 cM of mapping distance (Liu et al., 2009), the QTLs associated with GR were identified by composite interval mapping (CIM) of Windows QTL Cartographer 2.5 software (Wang et al., 2007). 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 (α = 0.05) (Doerge and Churchill, 1996). The QTLs with $\log_{10}$ of odds ratio (LOD) 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 curve region, then, the identified QTLs were mapped with Mapchart 2.1 software (Voorrips, 2002).

RESULTS

Phenotypic observation and statistic analysis

The results investigated on GR showed that the tested lines presented variations. For the three lines, Mo17, Huangzao4 and F_1, under HNR, F_1 had the highest values, followed by Huangzao4 (Table 1), while under LNR, Mo17 possessed higher value than F_1 while Huangzao4 presented the least GR. The results could be explained by ecological condition affecting same gene expression. With regard to the RIL population, the valid 236 RILs under HNR and LNR provided significant differences in GR at 0.01 probability level, respectively (Table 2). Nevertheless, the two group data presented significant positive correlation at 0.01 probability level (r = 0.9).

The results of the descriptive statistics for the RIL population are displayed in Table 3. The four values including range, standard deviation (SD), coefficient of variation (CV) and skewness under HNR were higher than those under LNR, while, the other four values including minimum, maximum, mean and kurtosis had contrary statistics results. From the frequency distribution graphs of the RIL population under two N regimes (Figures 1 and 2), it was found that the two group data could not agree well with normal distribution, which suggested that the trait GR were probably controlled by few genes in maize.

QTL mapping

Permutation test showed that the LOD threshold should be set at 2.58 and 2.65 for QTL identification under HNR and LNR, respectively. According to the LOD threshold values, three QTLs were detected under the two N regimes (Figures 3 and 4), of which the two QTL

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

N regime	Variation source	Sum of squares	df[a]	Mean square	F	Sig.
High N	Between groups	5.863	235	0.025	9.026**	＜0.01
	Within groups	1.305	472	0.003		
Low N	Between groups	4.006	235	0.017	12.052**	＜0.01
	Within groups	0.668	472	0.001		

[a]There were 3 missing values among the RIL population consisting of 239 RILs. **Significant difference at 0.01 probability level.

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

N regimes	Range	Minimum	Maximum	Mean	SD[a]	CV[b]	Skewness	Kurtosis
High N	0.500	0.400	0.900	0.781	0.091	0.117	-1.858	3.530
Low N	0.451	0.458	0.909	0.815	0.075	0.093	-2.227	5.472

[a] Standard deviation; [b] coefficient of variation.

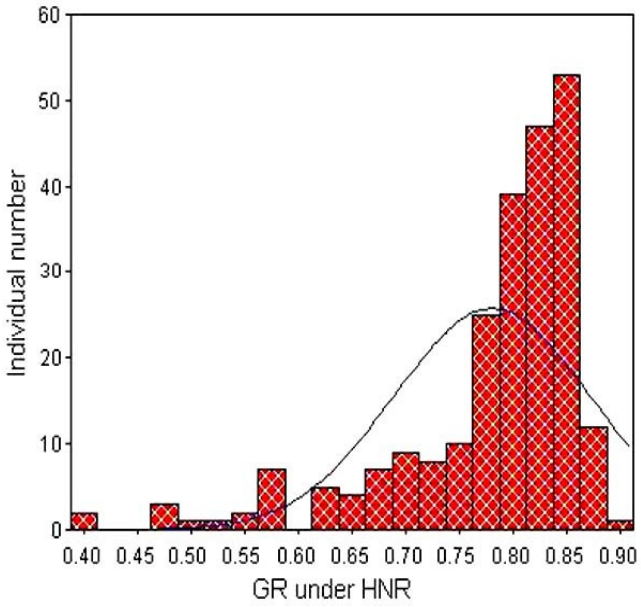

Figure 1. Frequency distribution of RIL population for GR under high N regime.

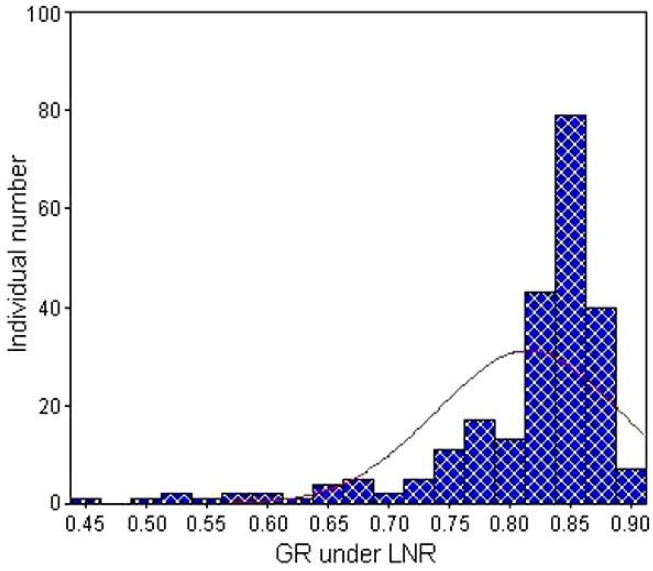

Figure 2. Frequency distribution of RIL population for GR under low N regime.

(named *Qgr-hn-1* and *Qgr-hn-2*) identified under HNR were located on chromosomes 6 and 9 and were identified under HNR, near to Umc1490 and Umc1893 (Figure 5 and Table 4), with 8.5 and 0 cM of mapping distance, respectively. The two QTLs could explain a total of 14.84% of phenotypic variance, decrease of 0.029 and increase of 0.0203 of GR due to additive effects. Under low N regime, only one QTL (named *Qgr-ln-1*) was identified on chromosome 6, near to Y1ssr, with 10 cM of mapping distance apart (Figure 5 and Table 4). This QTL could account for 9.52% of phenotypic variance and cause GR decrease of 0.0234 because of its additive effect.

DISCUSSION

GR is a very important agronomic trait in maize breeding project. To realize its genetic basis, in this study, a RIL population consisting of 239 RILs was used to identify the QTLs associated with GR under two N regimes. As a result, two QTLs (*Qgr-hn-1* and *Qgr-hn-2*) were located on chromosomes 6 and 9 under HNR, while under LNR, only one QTL (*Qgr-ln-1*) was mapped on chromosome 6. According to the phenotypic data of parental lines and the additive effects of these QTLs, it was concluded that *Qgr-hn-1* was from Mo17, while, *Qgr-hn-2* and *Qgr-ln-1* were from Huangzao4.

Compared to previous reports on QTL mapping for GR

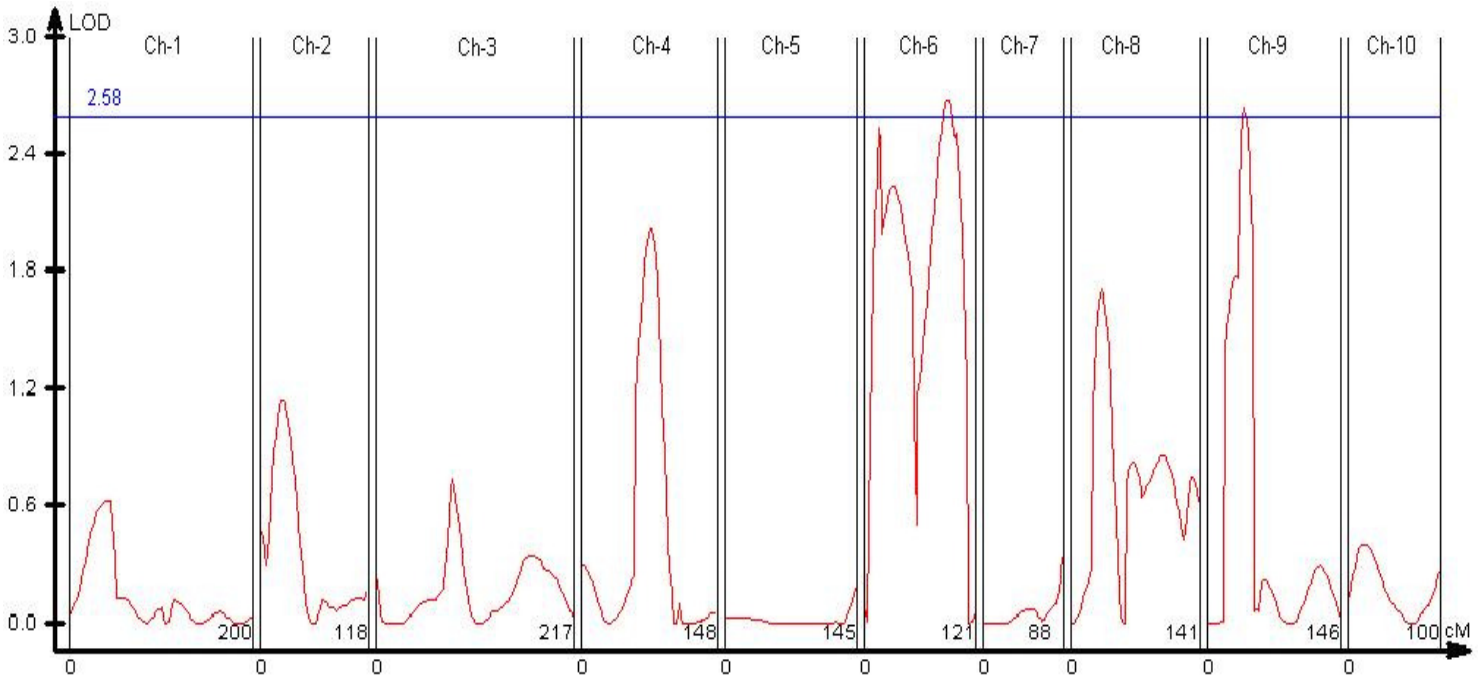

Figure 3. QTL scanning associated with GR using RIL population from the cross between Mo17 and Huangzao4 under high N regime. Two QTLs were detected on chromosomes 6 and 9 in a condition where 2.58 was set as QTL significance threshold.

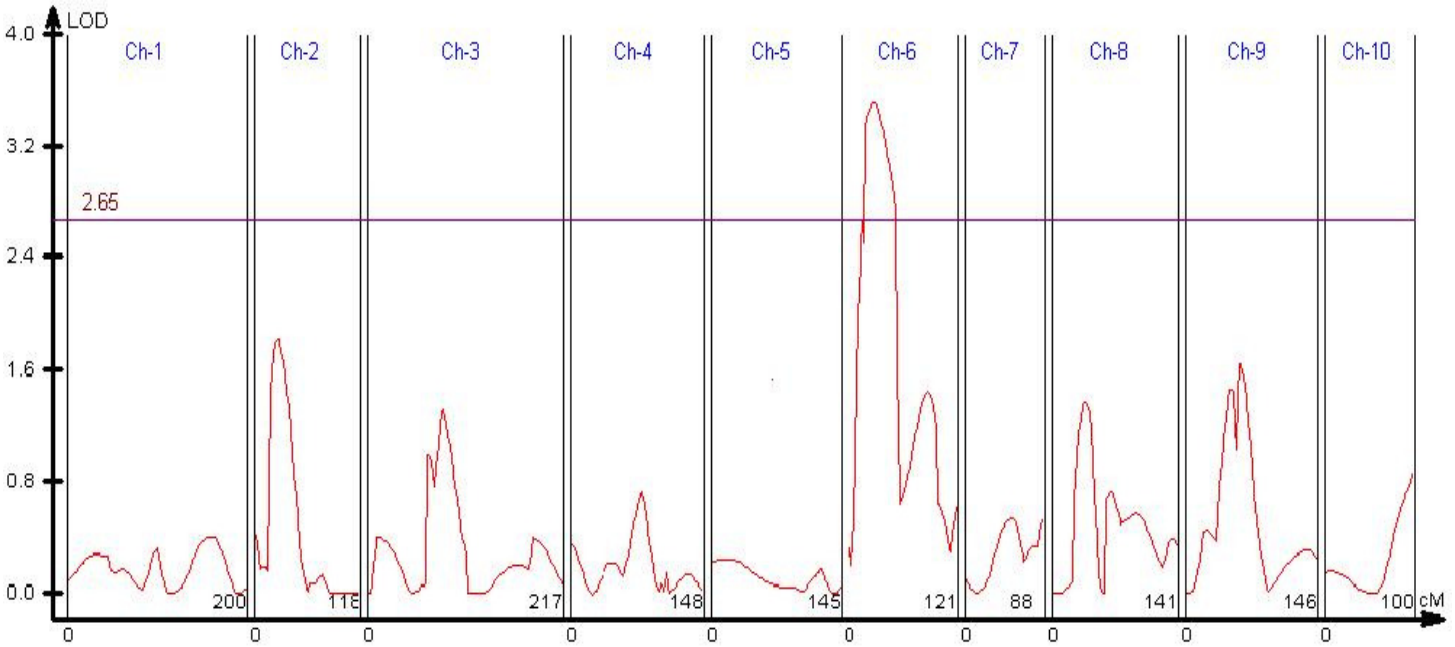

Figure 4. QTL scanning associated with GR using RIL population from the cross between Mo17 and Huangzao4 under low N regime. One QTL was identified on chromosome 6 in a condition where 2.65 was set as QTL significance threshold.

(Yang et al., 2005, Li et al., 2009), our studies differ from theirs in many aspects, and the main differences were listed in Table 5. For chromosomes 6 and 9, no QTLs were detected in their results, while, three QTLs were mapped in our experiments. Thus, the three QTLs reported in this present study were new loci associated with GR in maize. These differences of QTL mapping could probably result from different parental lines, segre-

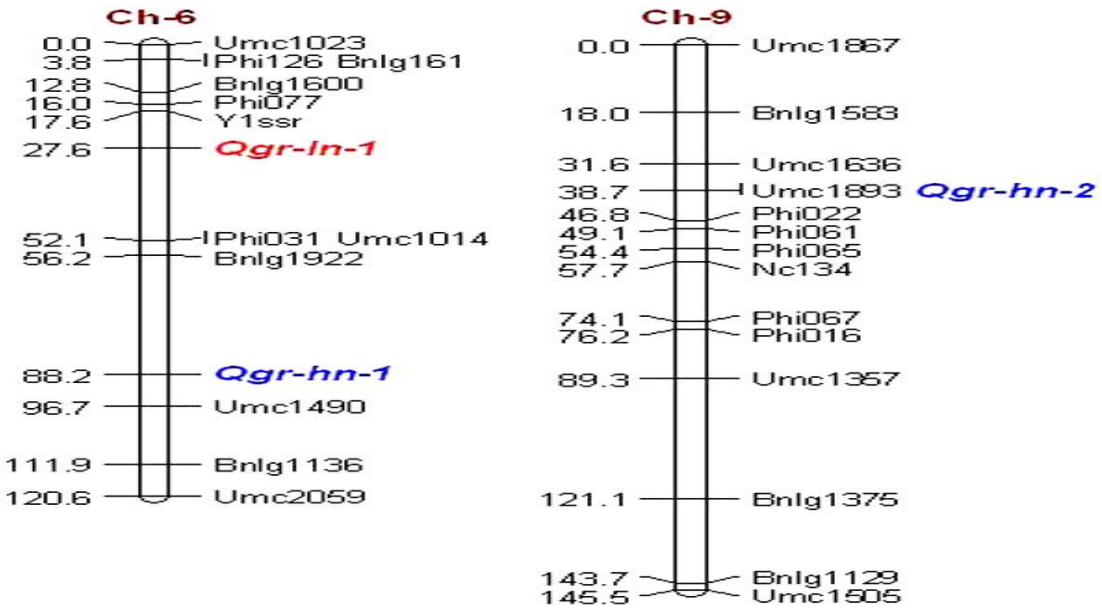

Figure 5. Chromosomal position of the QTLs for GR identified using RIL population under two N regimes, *Qgr-hn-1* and *Qgr-hn-2* under HNR (blue) and *Qgr-ln-1* under LNR (red).

Table 4. Positions and effects of the QTLs for GR identified under two N regimes.

N regime	QTL	Chr.	Flanking marker	Position (cM)	LOD[a]	R^2 (%)[b]	Additive effect
High N	*Qgr-hn-1*	6	Bnlg1922-Umc1490	88.2	2.68	9.95	-0.029
	Qgr-hn-2	9	Umc1893	38.7	2.63	4.89	0.0203
Low N	*Qgr-ln-1*	6	Y1ssr-Phi031	27.6	3.51	9.52	-0.0234

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

Table 5. The QTLs for GR identified in maize.

Reference	Parental line	Population	QTL number	Chromosome No.
Yang et al., 2005	48-2, 5003	F_2	5	1, 1, 2, 5 and 8
Li et al., 2009	Qi319, Huangzao4	F_2	2	3 and 5
This study	Mo17, Huangzao4	RIL	3	6, 6 and 9

gation populations or ecological conditions.

Moreover, the QTL on chromosome 9 in our study (named *Qgr-hn-2*) were quite near to marker Umc1893 (bin9.02), with only 0 cM of mapping distance between them. This suggested that Umc1893 could probably be co-segregated with the gene controlling GR within the QTL; thus, the marker could be considered in marker-assisted selection breeding, similar to previous reports (Wu et al., 2007; Guo et al., 2008). The other two QTLs (*Qgr-hn-1* and *Qgr-ln-1*) were far away from their linked markers, up to 8.5 and 10 cM, respectively. Nevertheless, other molecular markers could be added to map these QTLs more finely based on the constructed RIL population and genetic map, and the further work is in progress

In summary, a RIL population and different N regimes were first used to detect the QTLs for GR. The results showed that three QTLs were mapped on chromosomes 6 and 9. Of which the two QTLs identified under HNR could explain a total of 14.84% of phenotypic variance, and due to additive, the QTL on chromosome 6 from Mo17 could decrease to 0.029 of GR, while, the other QTL on chromosome 9 from Huangzao4 could cause a GR increase of 0.0203. The QTL on chromosome 6 identified under LNR from Huangzao4, could account for 9.52% of phenotypic variance and decrease of 0.0234 of GR due to its negative additive effect. The results of comparison with previous reports showed that the three QTLs in this present study were new loci associated with GR in maize. These results are beneficial for realizing the genetic basis of GR in maize breeding program.

ACKNOWLEDMENT

This work was financially assisted by Sichuan Key Subject Program (SZD0420), People's Republic of China.

REFERENCES

An D, Su J, Liu Q, Zhu Y, Tong Y, Li J, Jing R, Li B, Li Z (2006). Mapping QTLs for nitrogen uptake in relation to the early growth of wheat (*Triticum aestivum* L.). Plant Soil, 284: 73-84.

Ding JQ, Wang XM, Chander S, Li JS (2008). Identification of QTL for maize resistance to common smut by using recombinant inbred lines developed from the Chinese hybrid Yuyu22. J. Appl. Genet. 49: 147-154.

Doerge DW, Churchill GA (1996). Permutation tests for multiple loci affecting a quantitative character. Genetics, 142: 285-294.

Geffroy V, Sévignac M, De Oliveira JC, Fouilloux G, Skroch P, Thoquet P, Gepts P, Langin T, Dron M (2000). Inheritance of partial resistance against *Colletotrichum lindemuthianum* in Phaseolus vulgaris and co-localization of quantitative trait loci with genes involved in specific resistance. Mol. Plant Microbe. Interact. 13: 287-296.

Guo JF, Su GQ, Zhang JP, Wang GY (2008). Genetic analysis and QTL mapping of maize yield and associate agronomic traits under semi-arid land condition. Afr. J. Biotechnol. 7: 1829-1838.

Li YX, Wang Y, Shi YS, Song YC, Wang TY, Li Y (2009). Correlation analysis and QTL mapping for traits of kernel structure and yield components in maize. Sci. Agric. Sinica, 42: 408-418.

Liu XH, Tan ZB, Rong TZ (2009). Molecular mapping of a major QTL conferring resistance to SCMV based on immortal RIL population in maize. Euphytica, 167: 229-235.

Liu ZH, Tang JH, Wei XY, Wang EL, Tian GW, Hu YM, Chen WC (2007). QTL mapping of ear traits under low and high nitrogen conditions in Maize. Sci. Agric. Sinica, 40: 2409-2417.

Liu ZH, Xie HL, Tian GW, Chen SJ, Wang CL, Hu YM, Tang JH (2008). QTL mapping of nutrient components in maize kernels under low nitrogen conditions. Plant Breed. 127: 279-285.

Ribaut JM, Fracheboud Y, Monneveux P, Banziger M, Vargas M, Jiang C (2007). Quantitative trait loci for yield and correlated traits under high and low soil nitrogen conditions in tropical maize. Mol. Breed. 20: 15-29.

Ribaut JM, Jiang C, Gonzalez-de-Leon D, Edmeades GO, Hoisington DA (1997). Identification of quantitative trait loci under drought conditions in tropical maize. Yield components and marker-assisted selection strategies. Theor. Appl. Genet. 94: 887-896.

Sabadin PK, Souza, JrCL, Souza AP, Garcia AAF (2008). QTL mapping for yield components in a tropical maize population using microsatellite markers. Hereditas, 145: 194-203.

Sala RG, Andrade FH, Camadro EL, Cerono JC (2006). Quantitative trait loci for grain moisture at harvest and field grain drying rate in maize (*Zea mays*, L.). Theor. Appl. Genet. 112: 462-471.

Shan YH, Wang YL, Pan XB (2005). Mapping of QTLs for nitrogen use efficiency and related traits in rice (*Oryza sativa* L.). Scientia Agricultura Sinica, 4: 721-727.

Voorrips RE (2002). MapChart: Software for the graphical presentation of linkage maps and QTLs. J. Hered. 93: 77-78.

Wan XY, Wan JM, Jiang L, Wang JK, Zhai HQ, Weng JF, Wang HL, Lei CL, Wang JL, Zhang X, Cheng ZJ, Guo XP (2006). QTL analysis for rice grain length and fine mapping of an identified QTL with stable and major effects. Theor. Appl. Genet. 112: 1258-1270.

Wang S, Basten CJ, Zeng ZB (2007). Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm)

Wu JY, Ding JQ, Du YX, Xu YB, Zhang XC (2007). Genetic analysis and molecular mapping of two dominant complementary genes determining resistance to sugarcane mosaic virus in maize. Euphytica, 156: 355-364.

Xiang DQ, Cao HH, Cao YG, Yang JP, Huang LJ, Wang SC, Dan JR (2001). Construction of a genetic map and location of quantitative trait loci for yield component traits in maize by SSR markers. Acta Genet. Sinica, 28: 778-784.

Yang JP, Rong TZ, Xiang DQ, Tang HT, Huang LJ, Dai JR (2005). QTL Mapping of quantitative traits in maize. Acta Agron. Sinica, 31: 188-196.