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*Full Length Research Paper*

# Quantitative trait locus (QTL) mapping for 100-kernel weight of maize (*Zea mays* L.) under different nitrogen regimes

**Xiao-Hong Liu[1], Zu-Ping Zheng[2]*, Zhen-Bo Tan[3], Zhong Li[2] and Chuan He[2]**

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

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**100-kernel weight (KW) is one of the most important agronomic traits in maize (*Zea mays* L.), related to yield. To realize its genetic basis, in this study, a recombinant inbred line (RIL) population derived from the cross between Mo17 and Huangzao4 was used for quantitative trait locus (QTL) mapping for KW under high and low nitrogen (N) regimes. As a result, five QTLs were identified on chromosomes 3, 4, 7 and 9, of which three were detected under both N environments, while the other two QTLs were respectively detected under high and low N regimes. These QTLs could explain phenotypic variance from 4.47 to 14.47%. Due to additive effects, the three QTLs from Mo17, including two on chromosome 3 and one on chromosome 4, could increase KW from 0.64 to 1.01 g, while the other two from Huangzao4 on chromosomes 7 and 9 could decrease KW from 0.62 to 1.07 g. These results are beneficial for understanding the genetic basis of KW and developing the markers linked with KW for marker-assisted selection breeding in maize.**

**Key words:** Maize (*Zea mays* L.), 100-kernel weight, quantitative trait locus (QTL), recombinant inbred line (RIL), nitrogen regime.

## INTRODUCTION

Nitrogen (N) stress condition presents a major source of yield loss in maize (*Zea mays* L.). Currently, most maize in the world are planted under N-deficiency 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 affects maize growth and development, and 100-kernel weight (KW) is also affected severely.

KW is a very important trait considered necessary in maize breeding program and related to yield, but at present, the maize resources with high KW are quite lacking. To resolve this problem, an effective solution is to utilize useful genes associated with high KW to improve the trait, but, this first needs our understanding of its genetic basis. Quantitative trait locus (QTL) mapping is an efficient approach to realize genetic basis of trait, some QTLs controlling KW have been reported in maize (Lan et al., 2005; Liu et al., 2007; Wang et al., 2007a; Guo et al., 2008; Tang et al., 2010).

Although some QTLs associated with KW were already mapped, different parental lines, segregation population or ecological condition could lead to different results, including QTL number and location or effect. For example, using $F_2$ segregation population derived from the cross between Mo17 and Huangzao4, Lan et al. (2005) identified 4 QTLs on chromosomes 1, 2, 3 and 9 under Beijing city, People's Republic of China, while under Xingjiang Province, People's Republic of China, a total of 6 QTLs

*Corresponding author. Email: zzp0817@163.com.

**Abbreviations: KW,** 100-kernel weight; **CIM,** composite interval mapping; **CV,** coefficient of variation; **HNR,** high nitrogen regime; **LNR,** low nitrogen regime; **LOD,** $\log_{10}$ of odds ratio; **MAS,** marker-assisted selection; **QTL,** quantitative trait locus; **$R^2$,** percentage of phenotypic variance explained by QTL; **RIL,** recombinant inbred line.

**Table 1.** The phenotypic values of parental lines and $F_1$ hybrid in KW.

| N regime | Mo17 | Huangzao4 | $F_1$ hybrid |
|---|---|---|---|
| HNR | 27.08 | 25.40 | 31.83 |
| LNR | 26.13 | 24.08 | 30.33 |

HNR, High nitrogen regime; LNR, low nitrogen regime.

**Table 2.** The ANOVA test of the RIL population on KW under two N regimes.

| N regime | Variation sources | Mean square | F | Sig. |
|---|---|---|---|---|
| HNR | Between groups | 22.34 | ***6.92 | <0.001 |
| | Within groups | 3.227 | | |
| LNR | Between groups | 23.63 | ***7.60 | <0.001 |
| | Within groups | 3.11 | | |

***Significant difference at 0.001 probability level.

dard deviation (SD), coefficient of variation (CV) and were detected on chromosomes 3, 4, 5, 5, 6 and 9. Therefore, it is necessary and significant that different parental lines and population or ecological condition are used for identifying the QTLs controlling KW.

In this study, an $F_9$ recombinant inbred line (RIL) population and two N regimes were used to map the QTLs for KW in maize. The objectives are to: (1) Identify and compare the QTLs for KW and (2) look for the markers used for marker-assisted selection (MAS) in maize breeding project.

## MATERIALS AND METHODS

### Plant materials

The plant materials in this study included maize inbred lines Mo17 and Huangzao4, an $F_1$ hybrid and an $F_9$ RIL population consisting of 239 RILs. Mo17 and Huangzao4 are the representative lines of Lancaster and Tansipingtou heterotic groups, respectively, the $F_1$ hybrid and RIL population were derived from the cross between Mo17 and Huangzao4.

### Field experiments

At the experimental field of Nanchong Institute of Agricultural Sciences, Nanchong City, People's Republic of China, the 242 lines mentioned above were sown in a complete randomized design, with six replicates per line and 15 plants per replicate, single-plant planting and one ear per plant were designed as one replicate. As for the six replicates per 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.

At the time of harvest, the middle eight plants of every replicate of each line were individually investigated on the trait KW. Based on the investigated data of the RIL population, SPSS11.5 software was performed on descriptive statistics, analysis of variance (ANOVA) and correlation analysis for the trait KW.

### QTL mapping

Based on the phenotypic data of the RIL population and the genetic map consisting of 100 SSR markers (Liu et al., 2009), the QTLs for KW were detected by composite interval mapping (CIM) of Windows QTL Cartographer 2.5 software (Wang et al., 2007b), 2.0 cM and log10 of odds ratio (LOD) 2.5 were set as the walk speed and QTL significance threshold, respectively. Control parameters included standard CIM model, 5 control markers, 10-cM window size and forward regression method. A limit of detection (LOD) value of 2.5 was considered as the significant threshold value for declaring a QTL (Qi et al., 1998; Gilliland et al., 2006). The QTLs with a LOD value greater than the threshold value was presented, and their positions, genetic effects and percentage of phenotypic variation were computed using the mapping software. Then, the identified QTLs were mapped with Mapchart 2.1 software (Voorrips, 2002).

## RESULTS

### Phenotypic observation

According to the phenotypic data, the trait KW presented variations among the 242 tested lines. For the three lines: Mo17, Huangzao4 and $F_1$ hybrid, $F_1$ hybrid had the highest values under both N regimes, followed by Mo17 (Table 1). $F_1$ hybrid displayed higher values than parental lines which could be explained by heterosis. Regarding the RIL population, the 239 RILs under HNR and LNR showed differences in KW at 0.001 probability level (Table 2). Nevertheless, the result of correlation analysis demonstrated positive correlation between the two sets of data at 0.001 probability level ( $r = 0.806$) .

The results of the descriptive statistics for the RIL population are displayed in Table 3. The four values of the RIL population under HNR, including minimum, maxi-

**Table 3.** The descriptive statistics of RIL population on KW under different N regimes.

| N regime | Minimum (g) | Maximum (g) | Range (g) | Mean (g) | SD | CV (%) | Skewness | Kurtosis |
|---|---|---|---|---|---|---|---|---|
| HNR | 10.50 | 29.92 | 19.42 | 21.07 | 2.73 | 12.95 | -0.199 | 1.114 |
| LNR | 10.47 | 27.93 | 17.46 | 21.10 | 2.81 | 13.30 | -0.081 | 0.656 |

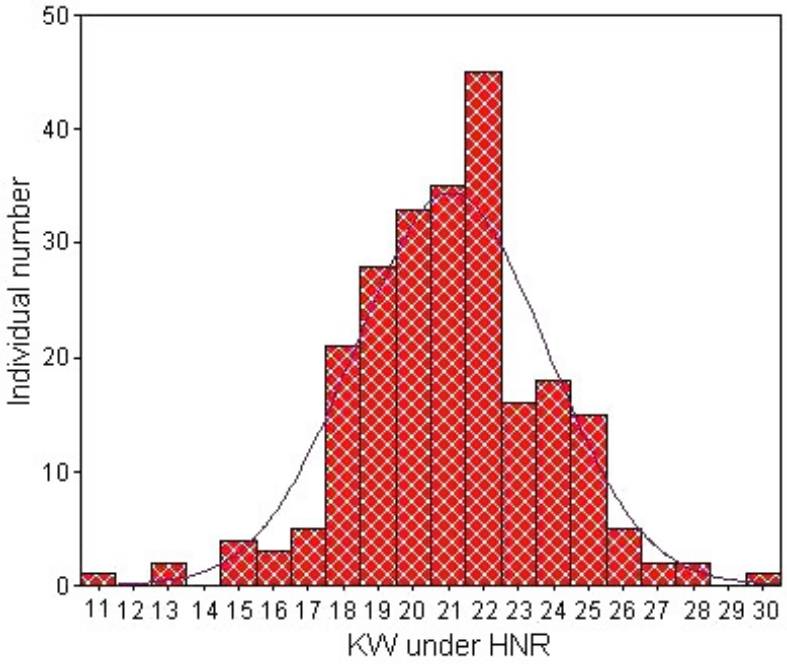


**Figure 1.** The frequency distribution of RIL population for KW under HNR.

mum, range and kurtosis, were higher than those under LNR, while the other four values, including mean, stan-skewness had contrary statistics results. Furthermore, from the frequency distribution graphs (Figures 1 and 2), the two groups of data could well agree with normal distribution, which meant that KW is a quantitative trait controlled by multiple genes.

## QTL mapping

Mapping software was performed to detect the QTLs for KW. As a result, under high N regime, four QTLs were detected on chromosomes 3, 4, 7 and 9 (Figures 3, 5 and Table 4), nearest to Umc1136, Umc2011, Phi112 and Umc1636, respectively; the mapping interval between these QTLs and the markers were 0, 3.3, 0 and 0 cM, respectively. The two QTLs on chromosomes 3 and 4 were from Mo17 and could increase 0.64 and 0.74 g of KW due to their positive additive effects while the other two QTLs on chromosomes 7 and 9 were from Huangzao4, and could decrease 0.70 and 0.78 g of KW, respectively due to their negative additive effects.

Under low N regime, similar results were obtained, there were also four QTLs identified on chromosomes 3, 4, 7 and 9, nearest to Umc2048, Umc2011, Phi112 and Umc1636, respectively (Figures 4, 5 and Table 4). Their mapping intervals between these QTLs and the markers were 12.0, 8.0, 0 and 2.0 cM, respectively. They could individually account for 12.63, 7.22, 4.47 and 14.47% of phenotypic variance. The two QTLs on chromosomes 3 and 4 were from Mo17 and could increase KW due to positive effects, while the other two QTLs on chromosomes 7 and 9 were from Huangzao4, and could decrease 0.62 and 1.07 of KW, respectively, owing to their negative effects.

## DISCUSSION

KW is a very important agronomic trait considered necessary in maize breeding project and related to yield. To realize its genetic basis, a RIL population was used to detect the QTLs controlling KW under two N regimes, and eight QTLs were identified under the two environments. However, from Figures 3, 4 and Table 4, it was concluded that *Qkw-hn-2* and *Qkw-ln-2*, *Qkw-hn-3* and *Qkw-ln-3*, *Qkw-hn-4* and *Qkw-ln-4* are the same QTLs,

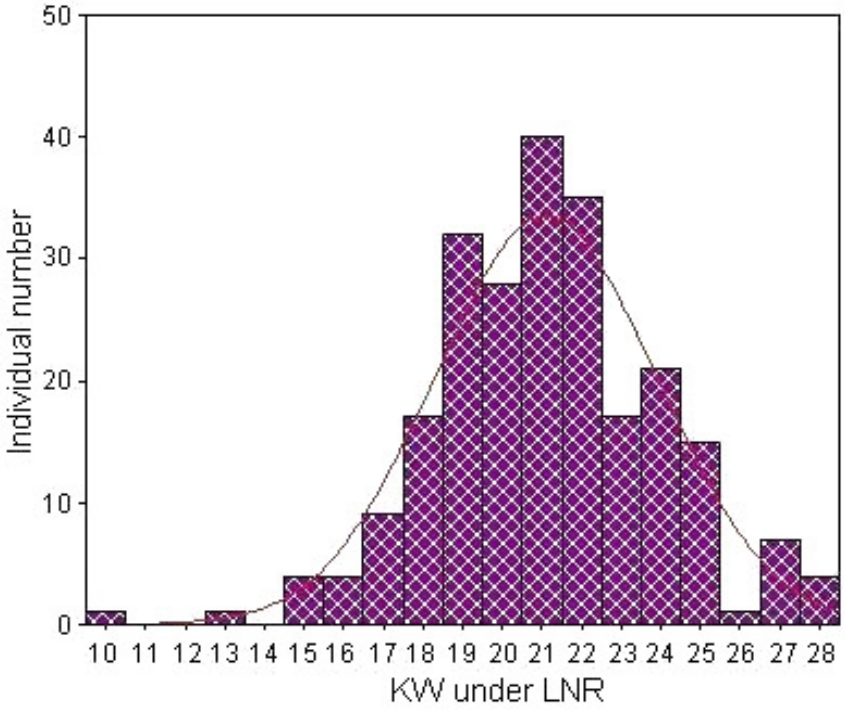


**Figure 2.** The frequency distribution of RIL population for KW under LNR.

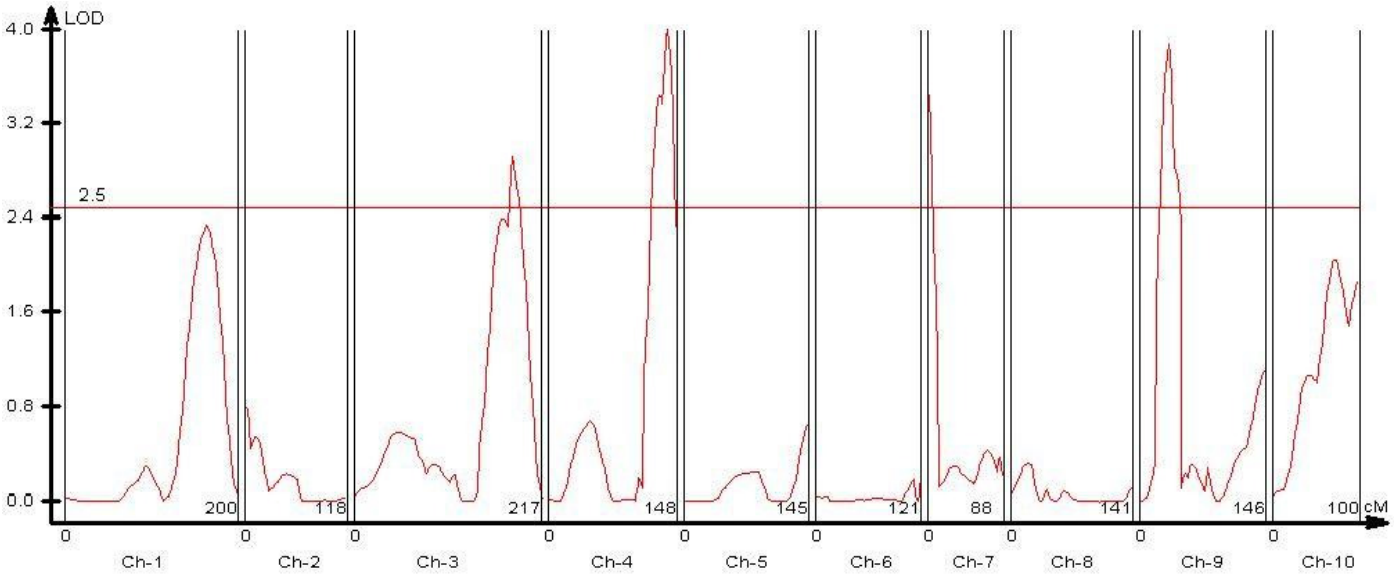


**Figure 3.** QTL scanning for KW under HNR by CIM. From the map, four QTLs were detected on chromosomes 3, 4, 7 and 9, in a condition where 2.5 was set as the LOD threshold value declaring QTLs.

respectively. So, only five QTLs for KW were detected in our study, of which three were observed under both N conditions.

Compared to the report by Liu et al. (2007), similar N environments were designed to map QTLs for KW, but our study was different from theirs in many aspects, and the main differences are listed in Table 5. Although QTL number reported by Liu et al. (2007) was more than ours, no QTL were located on chromosomes 3 and 4 in their study, while three QTLs were identified on the two chromosomes in our study. These differences, including QTL number and position between the two results, could

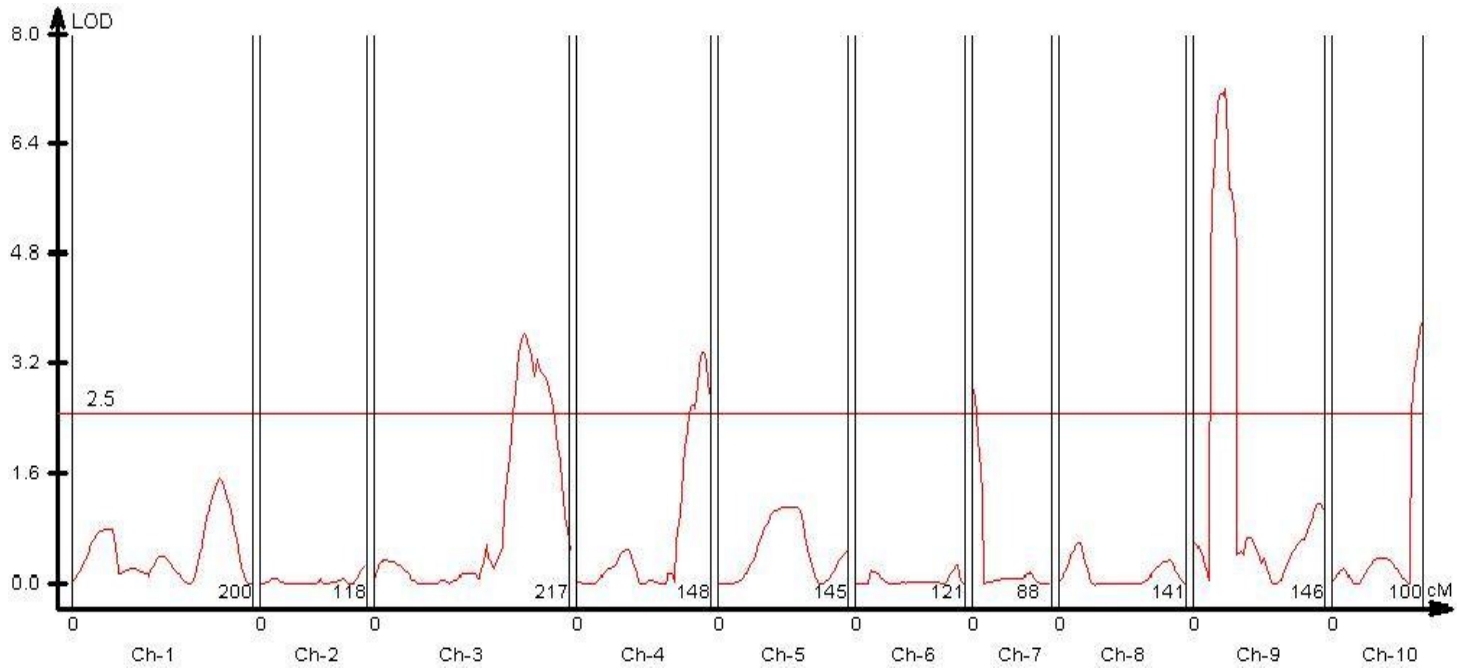


**Figure 4.** QTL scanning for KW under LNR by CIM. From the map, four QTLs were detected on chromosomes 3, 4, 7 and 9, in a condition where t 2.5 was set as the LOD threshold value declaring QTL.

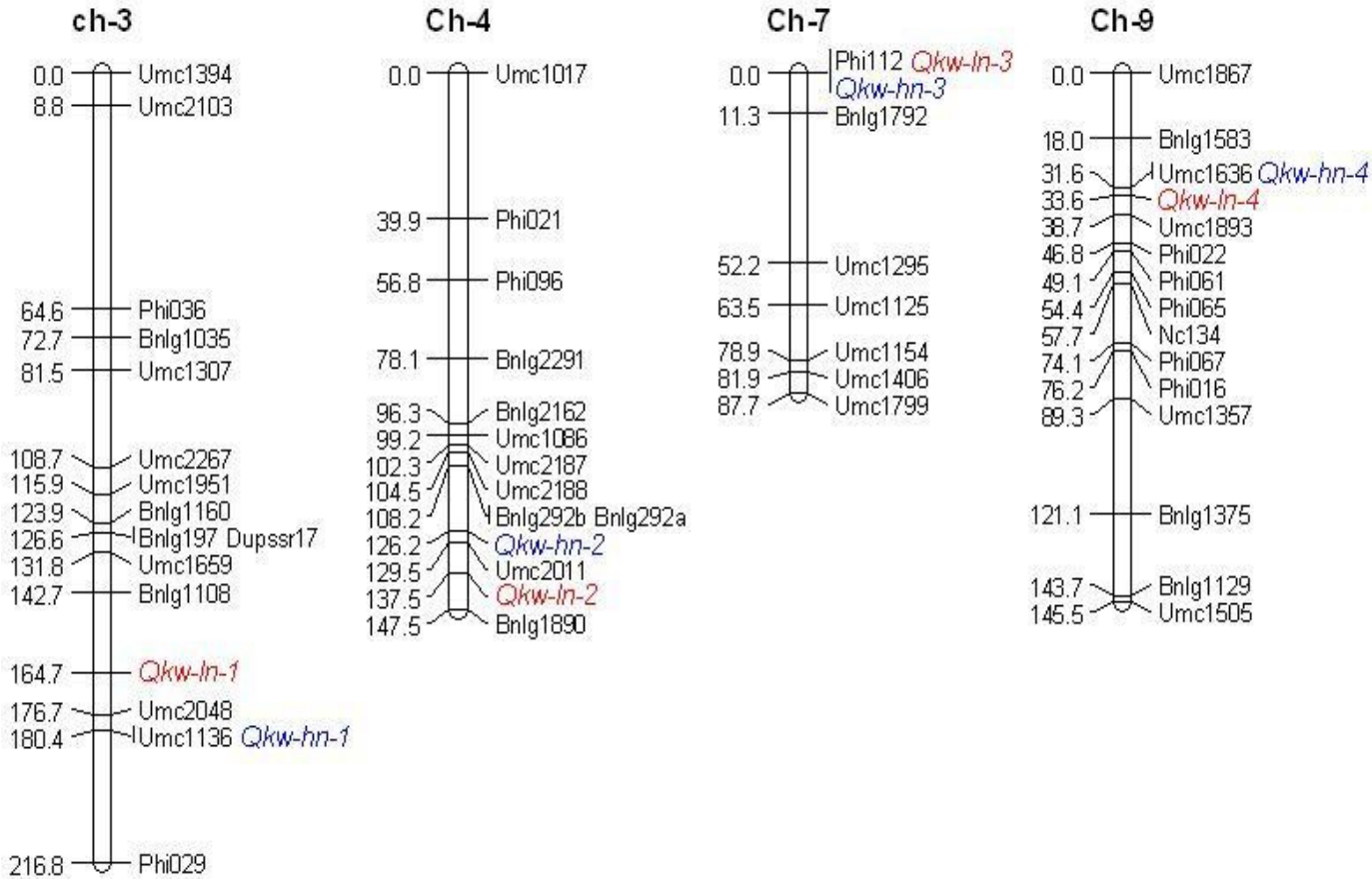


**Figure 5.** The chromosomal position of the QTLs for KW identified using RIL population under two N regimes. *Qew-hn-1*, *Qew-hn-2*, *Qew-hn-3* and *Qew-hn-4* under HNR (red), *Qew-ln-1*, *Qew-ln-2*, *Qew-ln-3* and *Qew-ln-4* under LNR (blue).

**Table 4.** The positions and effects of the QTLs associated with KW by CIM in maize.

| N regime | QTL | Chromosome | Position (cM) | Near marker (interval, cM) | LOD | $R^2$ (%) | Additive effect |
|---|---|---|---|---|---|---|---|
| High N | *Qkw-hn-1* | 3 | 180.4 | Umc1136 (0) | 2.93 | 5.01 | 0.64 |
| | *Qkw-hn-2* | 4 | 126.2 | Umc2011 (3.3) | 3.46 | 7.19 | 0.74 |
| | *Qkw-hn-3* | 7 | 0.0 | Phi112 (0) | 3.45 | 5.98 | -0.70 |
| | *Qkw-hn-4* | 9 | 31.6 | Umc1636 (0) | 3.89 | 8.13 | -0.78 |
| | Total | | | | | 26.32 | |
| Low N | *Qkw-ln-1* | 3 | 164.7 | Umc2048 (12.0) | 3.64 | 12.63 | 1.01 |
| | *Qkw-ln-2* | 4 | 137.5 | Umc2011 (8.0) | 3.38 | 7.22 | 0.76 |
| | *Qkw-ln-3* | 7 | 0.0 | Phi112 (0) | 2.83 | 4.47 | -0.62 |
| | *Qkw-ln-4* | 9 | 33.6 | Umc1636 (2.0) | 7.21 | 14.47 | -1.07 |
| | Total | | | | | 38.79 | |

**Table 5.** The QTLs for KW identified under different N environments.

| Reference | Parents | Population | Environment | QTL number (chromosome) |
|---|---|---|---|---|
| Liu et al. (2007) | HuangC Xu178 | $F_2$ | High N | 6 (5, 6, 7, 8, 9 and 10) |
| | | | Low N | 6 (1, 5, 5, 8, 9 and 1) |
| Lan et al. (2005) | Mo17, Huangzao4 | $F_2$ | Beijing, People's Republic of China | 4 (1, 2, 3 and 9) |
| | | | Xingjiang, People's Republic of China | 6 (3, 4, 5, 5, 6 and 9) |
| This study | Mo17, Huangzao4 | RIL | High N | 4 (3, 4, 7 and 9) |
| | | | Low N | 4 (**3**, 4, 7 and 9) |

probably be explained by the differences of parental lines, segregation population or genetic map.

Besides our study, the two parental lines Mo17 and Huanzao4 were also selected by Lan et al. (2005) to detect the QTLs for KW. Nevertheless, the differences were found in many respects (Table 5). For chromosomes 1, 2 and 5, a total of four QTLs were identified by them, while in our study, no QTLs was detected. With regard to chromosome 7, they could not locate any QTL, but we mapped one QTL. Moreover, the two QTLs (within bin3.08 and 3.10) on chromosome 3 in our study were different from the QTL reported (within bin3.05 and 3.06) despite the same chromosome. All the differences could be due to different segregation population, genetic map or ecological conditions.

The trait KW was also studied by some other researchers using different parental lines or environments (Guo et al., 2008; Wang et al., 2007a; Tang et al., 2010; Xiao et al., 2007), but, differences were found in QTL number, position and effect among these studies. The previous segregation populations were focused on $F_2$ (Lan et al., 2005; Liu et al., 2007; Tang et al., 2010; Wang et al., 2007a); this kind of population is temporary and could not be reused due to the fact that there is no continued plants, for it could not supply continued plants for phenotypic and genetic analysis (Pilet et al., 2001). Whereas, in this work, a RIL population was applied for QTL mapping, this kind of population is immortal and could be utilized again and again, this is similar to the previous reports by Tang et al. (2010) and Trachsel et al. (2010).

In summary, the QTLs of maize for KW were identified using RIL population under two N regimes. As a result, five QTLs were mapped on chromosomes 3, 4, 7 and 9, of which three were simultaneously under two N environments. These QTLs were close to their adjacent markers, with the intervals of 0 to 12 cM between them, and this could explain phenotypic variance from 4.47 to 14.47%. In addition, the three QTLs from Mo17, two on chromosome 3 and one on chromosome 4, could increase KW from 0.64 to 1.01 g, while the other two from Huangzao4 on chromosomes 7 and 9 could decrease KW from 0.62 to 1.07 g. These results are beneficial for understanding genetic basis of KW and developing the markers linked with KW for MAS in maize breeding program.

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