Research Paper Open Access

Characterization of a major quantitative trait locus on chromosome five for hundred-kernel weight of maize (*Zea mays* L)

Shunyou Wang[1], Chunxia Chen[1], Yuanyuan Feng[1], Qinghe Bai[1], Xue Xia[1], Xi Wang[1], Chenyu Ma[1], Qingli Zhou[1], Mingyang Lu[1], Zhangying Xi[1]*

[1]College of Agronomy, Henan Agricultural University, Zhengzhou 450002, China
*Corresponding author: E-mail: xizhangying@163.com

Abstract

Kernel weight is one of the most important components of grain yield and is controlled by quantitative trait loci (QTLs) derived from natural variations in maize. However, the molecular roles of QTLs in the regulation of kernel weight have not been fully elucidated. In this study, by using homozygous chromosome single segment substitution lines Z22(SSSL-Z22) as base material, two $F_{2:3}$ populations derived from a cross between elite maize inbred line Zheng58 and SSSL-Z22, were employed to map QTLs of kernel weight traits in two years at the same location. Out of four traits, 3 QTLs were detected in one of the two environments whereas 2 detected in both environments. Two major QTLs, *qhkw5-3* for hundred-kernel weight and *qkw5-3* for kernel width, were consistently detected in similar chromosome segment in different years. *qhkw5-3* was mapped to Bin 5.06 flanked by the SSR markers SYM033 and SYM108 with a genetic interval of 8.8 cM, which made kernel size smaller. *qkw5-3* was identified between SYM024 and SYM129 with a genetic interval of 13.9 cM. These results will help to promote the fine mapping and cloning of the target gene and further develop linked markers to be used in marker-assisted breeding.

Keywords: maize, single segment substitution line, major QTL, hundred kernel weight, kernel width

Introduction

Maize is one of the most important food, feed and economic crops in the world. The research on genetic control mechanisms of grain yield and yield-associated traits has always been focused by maize research scientist. A lot of maize yield quantitative trait loci (QTLs) were mapped in the past (Doebley et al, 1994; Peng et al, 2010; Messmer et al, 2009; Berke et al, 1995; Goldman et al, 1994; Schön et al, 1994; Veldboom and Lee, 1994; 1996; Yan et al, 2006; Xiang et al, 1999; 2000; 2001; Yang et al, 1999; 2004; Zhou et al, 2015; Wang et al, 2012; Chen et al, 2013). Partial genetic mechanisms of some maize yield-associated traits were explained in different experiments.

Several kinds of mapping populations were often used in QTL analysis, such as the primary mapping population F_2 (Doebley et al, 1994), S_1 (Berke et al,1995; Goldman et al, 1994), $F_{2:3}$ (Peng et al, 2010; Veldboom et al, 1994; 1996; Yan et al, 2006; Xiang et al, 2000; 2001; Yang et al, 2004), BC_1 (Schön et al, 1994), RIL (Messmer et al, 2009), and the secondary mapping population such as CSSL (Zhou et al, 2015). With the development of technology, the chromosome single segment substitution lines (SSSLs) (Wang et al, 2012; Chen et al, 2013) were also utilized. A major QTL on grain yield and yield-related traits was detected on chromosome 5 by Stuber et al (1992), using a maize (*Zea mays* L) population generated from the cross B73 × Mo17. A set of BC_2S_1 lines was created by Graham et al (1997), each containing an introgressed segment of Mo17 in a B73 background. The major QTL on chromosome 5 was dissected into at least two smaller QTLs near restriction fragment length polymorphism (RFLP) marker Amp3. Liu et al (2012) identified a major QTL, *qKNPR6*, for kernel number per row (KNPR) across multiple environments, and developed two near isogenic lines, SL57-6 and Ye478, which differ only in the allelic constitution of the short segment harboring the QTL. *qKNPR6* was re-evaluated in segregating populations derived from SL57-6 × Ye478, and was narrowed down to a 2.8 cM interval, which explained 56.3% of the phenotypic variance of KNPR in 201 $F_{2:3}$ families. A large F_2 population with more than 12,800 plants, 191 recombinant chromosomes and 10 overlapping recombinant lines placed *qKNPR6* into a 0.91 cM interval corresponding to 198 Kb of the B73 reference genome, flanked by simple sequence repeat (SSR) markers N6M19 and umc1257 on chromosome 6. One major QTL (named *qKN*), controlling kernel number (KN) under different phosphorus (P) regimes, was mapped to the interval between SSR molecular markers SSR15 and SSR19 on chromosome 10 using an $F_{2:3}$ population derived from the cross between maize inbreds 178 and 5003. The QTL *qKN* was finally localized in a region of 480 kb (Zhang et al, 2013) using a population of near isogenic lines (NILs).

A set of chromosome single-segment substitution lines (SSSLs) were developed from an advanced backcross procedure with the marker-assisted selection using the elite maize inbred line Zheng58 as the recipient and Chang7-2 as the donor. Each SSSL

 RECEIVED 2/06/2016

contained a single substituted chromosome segment that was derived from donor strain Chang7-2 in the genetic background of the recipient Zheng58 strain (Lu et al, 2012). Fifty-nine homozygous chromosome SSSLs were employed to identify the QTL of hundred kernel weight of maize under six environments, the major QTL *qhkw5-3* out of SSSL-Z22, which was detected repeatedly under six environments, was located near the SSR marker umc1680 (Chen et al, 2013). In this study, by using SSSL-Z22 as base material, *qhkw5-3* was re-evaluated in segregating populations derived from SSSL-Z22 × Zheng58, and we hope to narrow it down to a small interval, so as to lay a foundation for its fine mapping and map-based cloning.

Materials and Methods

Plant materials

SSSL-Z22 is a homozygous chromosome SSSL, which was developed from an advanced backcross procedure with the marker-assisted selection using the elite maize inbred line Zheng58 as the recipient and Chang7-2 as the donor. SSSL-Z22 only contained a single substituted chromosome segment bnlg278 - umc1680 - bnlg1306 that was derived from donor strain Chang7-2 in the genetic background of the recipient Zheng58 strain (Lu et al, 2012). The hundred kernel weight (HKW) of SSSL-Z22 was evaluated under six environments during two years. The HKW of SSSL-Z22 was significantly lower than that of the recurrent parent Zheng58's, indicating that a major QTL of HKW in target substituted fragment was identified, designated as *qhkw5-3* (Chen et al, 2013). Two $F_{2:3}$ segregating populations derived from SSSL-Z22 × Zheng58, containing 231 and 301 family lines, respectively, were employed to in-depth map the major QTL *qhkw5-3* for hundred kernel weight.

Phenotype evaluation

The $F_{2:3}$ populations and parental lines (Zheng58, SSSL-Z22, and Chang7-2) were planted at Sanya(18°25'47"N, 108°58'59"E), Hainan province, China, in 2011 and 2013, respectively. Each line was arranged according to partition-compares-method with single replication in the field plot. Zheng58 was planted as the field control at the interval of nine lines. Regular field management was maintained.

Twelve mature ears from each line were harvested and air-dried. Ten kernels in middle of each ear were picked up for the measurement of kernel length (KL), kernel width (KW) and kernel thickness (KT) using vernier caliper; the other kernels of each ear were used to evaluate kernels number per ear (KNE) and kernel weight per ear (KWE). Hundred kernel weight (HKW) was calculated with: HKW = KWE × KNE^{-1} × 100. The investigation method reference national standard (GB/T19557-2007): Guidelines for the conduct of tests for distinctness, uniformity and stability--Corn (*Zea mays* L).

Genotyping and QTL mapping

Genomic DNA was extracted from fresh seedling leaves using SDS method (Dellaporta et al, 1983). SSR primers used in this study (Supplementary Table 1) were designed based on B73 sequence using Primer Premier 6.0 (Premier Biosoft International, Palo Alto, CA, USA), and synthesized by Sangon Biotech Co, Ltd. (http://www.sangon.com).

DNA amplification was carried out in volumes of 8 µl, containing 3.8 µl of 2 × Taq PCR MasterMix (0.1 U Taq Polymerase $µl^{-1}$, 500 µM dNTP each, 20 mM Tris-HCl, 100 mM KCl, 3 mM $MgCl_2$ and other stabilizer and fortifier), 2 µM of primers, 2 µl of ddH_2O and 20 ng of DNA templates. Amplification was conducted on Biometra DNA Thermal Cycler with 5 min at 95°C initially, then 35 cycles of 50s at 95°C, 50s at 50-60°C for different primers, and 60s at 72 °C, and finally, 10 min at 72°C for a final extension. The products were separated on 6% polyacrylamide gels and visualized with silver staining. All marker data were scored by visual inspection and the ambiguous bands were scored as missing genotype. The polymorphic markers were used for genotyping of the $F_{2:3}$ population.

Analysis of variance (ANOVA) was performed using Microsoft Excel 2010 (Millar, 2001). The broad-sense heritability were calculated with formula: $H^2(\%) = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2 / r) \times 100\%$, where σ_g^2 is the genetic variance, σ_e^2 is the error variance, and r is the number of replicates (Hallauer and Miranda, 1988). The genetic linkage map (Supplementary Figure 1) was constructed using JoinMap 4.0 (Van Ooijen, 2011) according to the protocol of the software. The genetic distances were calculated with the Kosambi function. Window QTL Cartographer v2.5 (Wang et al, 2007) was used for QTL detection and QTL effects were estimated using the composite interval mapping (CIM) function. CIM Model 6 was selected using five control markers, a window size of 10 cM and the backward regression method. The threshold for log-likelihood (LOD) scores was set at 3.0 with a 1 cM walking speed, 500 permutations and a significance level of 0.05.

Results

Phenotypic analysis

Kernel weight and its components were obtained from field trials grown during 2011 and 2013. The results of variance analysis between parental strains SSSL-Z22 and Zheng58 demonstrated that significant differences were found for HKW and KW, and both of HKW and KW of SSSL-Z22 were smaller than Zheng58's.

The phenotypic values of four kernel size related traits HKW, KL, KW, and KT among 231 $F_{2:3}$ family lines ranged from 18.32 to 35.71 g, 8.55 to 11.22 mm, 7.60 to 9.36 mm, and 4.43 to 6.29mm, and their broad-sense heritability (H^2%) were 79.10%, 10.82%, 4.58%, and 4.40%, respectively, in 2011. Similarly, the corresponding values of HKW, KL, KW,

Table 1 - Performance of hundred-kernel weight (HKW), kernel length (KL), kernel width (KW), and kernel thickness (KT) for parents and $F_{2:3}$ family lines in two years.

Year	Trait	Parent			$F_{2:3}$ Population					
		SSSL-Z22	Zheng58		Min	Max	Aver	Kurt	Skew	H^2(%)
2011	HKW (g)	21.46±2.81	27.83±2.09	**	18.32	35.71	28.31	0.88	-0.27	79.10
	KL (mm)	9.62±0.67	10.28±0.25		8.55	11.22	10.34	0.22	-0.37	10.82
	KW (mm)	7.99±0.45	8.52±0.21	*	7.60	9.36	8.70	0.19	-0.20	4.58
	KT (mm)	6.30±1.07	7.29±0.25		4.43	6.29	5.21	0.32	0.28	4.40
2013	HKW (g)	26.38±0.24	32.79±1.95	**	25.00	36.99	30.94	-0.41	0.14	79.01
	KL (mm)	10.18±0.08	10.57±0.41		9.21	11.60	10.41	0.08	-0.11	8.95
	KW (mm)	8.49±0.17	9.33±0.22	**	7.98	9.72	8.90	-0.11	-0.05	5.78
	KT (mm)	5.54±0.55	5.40±0.27		4.67	6.87	5.39	1.96	0.91	7.01

*and ** indicate significant between Zheng58 and SSSL-Z22 at P = 0.05 and 0.01, respectively. H^2 (%) - broad-sense heritability.

and KT among 301 $F_{2:3}$ family lines ranged from 25.00 to 36.99 g, 9.21 to 11.60 mm, 7.98 to 9.72 mm, and 4.67 to 6.87 mm, and their broad-sense heritability (H^2%) were 79.01%, 8.95%, 5.78%, and 7.01%, respectively, in 2013 (Table 1).

Linkage analysis

Two $F_{2:3}$ segregation populations (231 and 301 families) were planted for mapping of four kernel size related traits in 2011 and 2013, respectively. Ten simple sequence repeat (SSR) markers (bnlg1847, P12, mmc0481, umc1680, phi087, P8, umc2306, P14, P9, and umc2201) were employed to map QTLs of HKW, KL, KW, and KT in 2011. Three QTLs (*qhkw5-3*, *qkw5-3*, and *qkt5-3*) for HKW, KW, and KT were identified in a similar genomic region of Bin 5.06 on chromosome 5. Their genetic intervals were 4.9cM, 4.5cM, and 7.4cM, flanked by mmc0481 and P8, umc1680 and umc2306, and P12 and P8, respectively. The percentages of phenotypic variance of these QTLs were 7.33% for HKW, 7.54% for KW and 11.20% for KT, respectively (Table 2).

Furthermore, we developed 28 newly SSR markers (Supplementary Table 1) in the target genomic region. A new $F_{2:3}$ population containing 301 family lines was employed to confirm these QTLs in 2013. The results demonstrated that two QTLs were re-identified in the new experiment. *qhkw5-3* was mapped to a genetic interval of 8.8 cM flanked by SSR markers SYM033 and SYM108, which explained 17.10% of the phenotypic variance. *qkw5-3* was mapped to 13.9 cM flanked by SSR marker SYM024 and SYM129, which explained 29.91% of the phenotypic variance (Table 2, Figure 1).

The mapping results of HKW, KL, KW, and KT showed differences across the two experiments (2011 and 2013), *qhkw5-3* and *qkw5-3* were consistently detected in similar chromosome segment, indicating that these two QTLs were stably expressed in different years. And the additive effects of these two QTLs were negative, which means they can regulate the width and weight of maize kernel.

Discussion

In this study, two mapping populations were employed to map QTLs of maize kernel size. Two major QTLs (*qhkw5-3*, *qkw5-3*) were consistently detected in similar chromosome segment in different years. *qhkw5-3* for hundred kernel weight was mapped in Bin 5.06 flanked by SYM033 and SYM108, which made kernel size smaller. *qkw5-3* controlled kernel width was mapped in the same genomic region. These results indicated that the decreasing of HKW of SSSL-Z22 might be caused by the kernel width narrowing. Whether *qhkw5-3* and *qkw5-3*, which controlled HKW and KW respectively, were the same gene should be subject to further verification.

About 887 QTLs for maize grain yield and its components have been identified (www.maizegdb.org/cgi-bin/qtl_loci_summary_table.cgi), and 15 QTLs were mapped in Bin 5.06 (Austin and Lee, 1998; Xiang et al, 2001; Lan et al, 2005; Lu et al, 2006; Yan et al, 2006; Peng et al, 2011; Li et al, 2011; Li et al, 2013). A major QTL for grain yield and yield related traits was detected on chromosome 5 by Graham et al (1997). Both Austin (1998) and Lu et al (2006) considered that the QTL for HKW near SSR marker umc1680 was major. Four hundred and fifty-eight genes for kernel traits have been mapped from maize mutant (Neuffer et al, 1995; 1997), three (*de*-N1196*, *o*-N1065A*, and *smk*-N1160*) of them could make

Table 2 - Interval, additive effects and contribution rate of QTL for kernel size-related traits in two years.

Year	n	QTL	Trait	Flanking markers	Genetic Interval(cM)	Physical Interval(Mb)	LOD	A	D	R^2(%)
2011	231	*qhkw5-3*	HKW	mmc0481-P8	4.9	6.20	3.81	-2.86	2.57	7.33
		qkw5-3	KW	umc1680-umc2306	4.5	2.10	3.96	-0.27	0.58	7.54
		qkt5-3	KT	P12-P8	7.4	7.11	6.13	0.84	1.90	11.20
2013	301	*qhkw5-3*	HKW	SYM033-SYM108	8.8	4.44	12.29	-3.23	1.48	17.10
		qkw5-3	KW	SYM024-SYM129	13.9	7.28	23.33	-0.46	-0.03	29.91

n - population size; HKW - hundred-kernel weight; KL - kernel length; KW - kernel width; KT - kernel thickness; LOD - log10 likelihood ratio; A - additive effect; D - dominant effect ; R^2(%) - contribution rate of QTL.

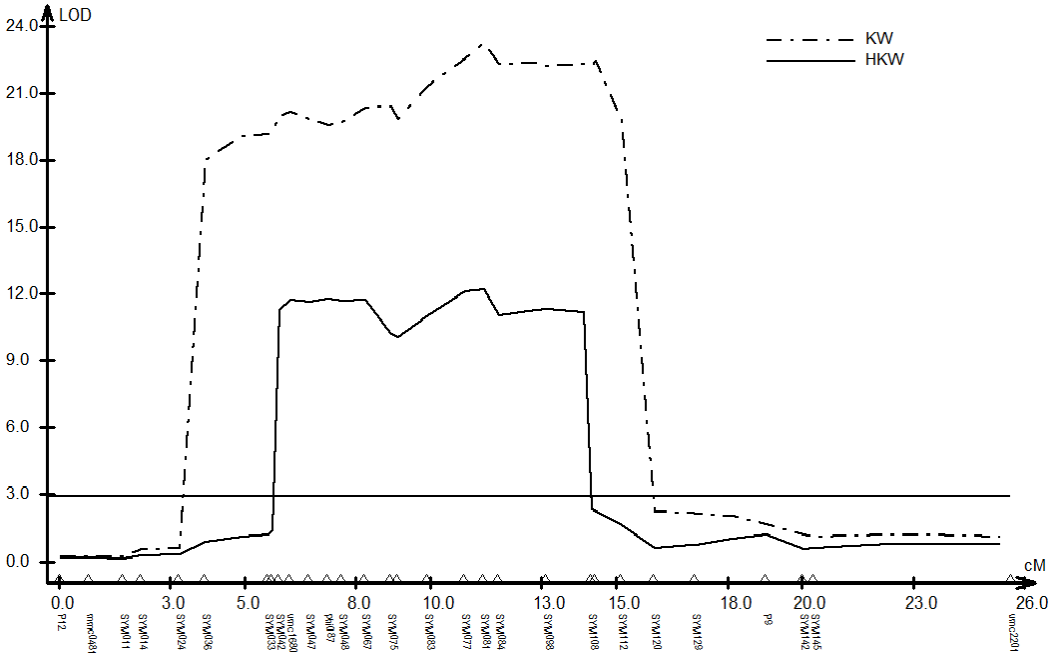

Figure 1- QTL likelihood curve of the LOD score for kernel size-related traits on chromosome 5 in 2013. The chromosome and associated SSR markers are listed on the x axis. The horizontal solid line indicates the LOD score for declaring putative QTL.

maize kernel smaller. *de*-N1196*, *o*-N1065A*, and *smk*-N1160* were located in the same chromosome region with *qhkw5-3* and *qkw5-3*. These research results indicated that a major gene controlling maize kernel size might exactly exist in the genomic region of Bin 5.06.

Bioinformatics analysis showed that there were 207 open reading frames (ORF) in the genomic region (SYM024 - SYM129) of Bin 5.06 (Schnable et al, 2009; Soderlund et al, 2009; Alexandrov et al, 2009). Eighty-nine ORFs had descriptions, and 7 ORFs effected kernel development. The functions of these 7 ORFs could be summarized in three categories (Table 3): growth-regulating factors (GRMZM2G034876, GRMZM2G045977), E3 ubiquitin ligases (GRMZM2G130167, GRMZM2G004480), and RING zinc finger protein (GRMZM2G071277, GRMZM2G057789, and GRMZM2G375153). OsGRF (Oryza sativa GROWTH-REGULATING FACTOR1) genes act as a transcriptional activator, and were preferentially expressed in young and growing tissues (Choi et al, 2004). In maize, 14 homologous GRF genes were expressed at all three stages of preparation for maize grain filling, active starch accumulation, and maximum starch synthesis by transcriptome sequencing performed on the developing maize kernels (Zhang et al, 2015). In Arabidopsis, two E3 ubiquitin ligases, DA2 and Big Brother (BB) / Enhancer of DA1 (EOD1), were identified as negative regulators of seed size(Li et al, 2008; Xia et al, 2013). Most of the RING type zinc finger protein with E3 ubiquitin ligase activity can be used as an E3 ubiquitin ligase involved in various physiological activities (Deshaies and Joazeiro, 2009). Song (2007) has successfully cloned a QTL, *GW2*, that controls rice grain width and weight. *GW2* encodes a new RING-type protein with E3 ubiquitin ligase activity, which is known to function in the degradation by the ubiquitin-proteasome pathway. Loss of *GW2* function increased cell numbers, resulting in a larger (wider) spikelet hull, and it accelerated the grain milk filling rate, resulting in enhanced grain width, weight and yield. *GW2* negatively regulates cell division by targeting its substrate(s) to proteasomes for regulated proteolysis. Which were the candidate genes of *qhkw5-3* and *qkw5-3* still need to further investigate a genetic complementation by transformation and other functional analysis.

Table 3 - List of categories in kernel size control.

Categories	Reference(s)	ORF	Marker interval
growth-regulating factors	OsGRF (Choi et al, 2004), GS2 (Lan et al, 2012)	GRMZM2G034876 GRMZM2G045977	SYM105-SYM108 SYM035-SYM042
E3 ubiquitin ligases	DA2 (Xia et al, 2013), EOD1/BB (Deshaies and Joazeiro, 2009), DA2L (Xia et al, 2013)	GRMZM2G130167 GRMZM2G004480	SYM084-SYM098 SYM084-SYM098
RING zinc finger protein	GW2 (Song et al, 2007), TaGW2 (Su et al, 2011; Bednarek et al, 2012; Yang et al, 2012), ZmGW2 (Li et al, 2010)	GRMZM2G071277 GRMZM2G057789 GRMZM2G375153	SYM035-SYM042 umc1680-phi087 SYM067-SYM077

Acknowledgements

This work was funded by the National Natural Science Foundation of China(31371629).

References

Alexandrov NN, Brover VV, Freidin S, Troukhan ME, Tatarinova TV, Zhang HY, Swaller TJ, Lu YP, Bouck J, Flavell RB, Feldmann KA, 2009. Insights into corn genes derived from large-scale cDNA sequencing. Plant Mol Biol 69: 179-194

Austin DF, Lee M, 1998. Detection of quantitative trait loci for grain yield and yield components in maize across generations in stress and nonstress environments. Crop Sci 38(5): 1296-1308

Bednarek J, Boulaflous A, Girousse C, Ravel C, Tassy C, Barret P, Bouzidi MF, Mouzeyar S, 2012. Down-regulation of the *TaGW2* gene by RNA interference results in decreased grain size and weight in wheat. J Exp Bot 63(16): 5945-5955

Berke TG, Rocheford TR, 1995. Quantitative trait loci for flowering, plant and ear height, and kernel traits in maize. Crop Sci 35(6):1542-1549

Chen CX, Lu MY, Shang AL, Wang YM, Xi ZY, 2013. Analysis of QTL for 100-kernel weight using chromosome single-segment substitution lines in maize. Acta Agron Sin 39(9): 1562-1568

Choi D, Kim JH, Kende H, 2004. Whole genome analysis of the OsGRF gene family encoding plant-specific putative transcription activators in rice (*Oryza sativa* L.). Plant Cell Physiol 45(7): 897-904

Dellaporta SL, Wood J, Hicks JB, 1983. A plant DNA minipreparation: version II. Plant molecular biology reporter 1(4): 19-21

Deshaies RJ, Joazeiro CAP, 2009. RING domain E3 ubiquitin ligases. Annual review of biochemistry 78: 399-434

Doebley J, Bacigalupo A, Stec A, 1994. Inheritance of kernel weight in two maize-teosinte hybrid populations: implications for crop evolution. J Hered 85(3): 191-195

Goldman IL, Rocheford TR, Dudley JW, 1994. Molecular markers associated with maize kernel oil concentration in an Illinois high protein × Illinois low protein cross. Crop Sci 34(4): 908-915

Graham GI, Wolff DW, Stuber CW, 1997. Characterization of a yield quantitative trait locus on chromosome five of maize by fine mapping. Crop Sci 37(5): 1601-1610

Hallauer AR, Carena MJ, Miranda Filho JB, 1988. Heredity variance: Mating design. Quantitative Genetics in Maize Breeding, 2nd edn. Iowa State University Press, Ames, Iowa

Lan JH, LI XH, Gao SR, Zhang BS, Zhang SH, 2005. QTL analysis of yield components in maize under different environments. Acta Agron Sin 31(10): 1253-1259

Li CH, Li YX, Sun BC, Peng B, Liu C, Liu ZZ, Yang ZZ, Li QC, Tan WW, Zhang Y, Wang D, Shi YS, Song YC, Wang TY, Li Y, 2013. Quantitative trait loci mapping for yield components and kernel-related traits in multiple connected RIL populations in maize. Euphytica 193(3): 303-316

Li JZ, Zhang ZW, Li YL, Wang QL, Zhou YG, 2011. QTL consistency and meta-analysis for grain yield components in three generations in maize. Theor Appl Genet 122(4): 771-782

Li Y, Zheng L, Corke F, Smith C, Bevan MW, 2008. Control of final seed and organ size by the DA1 gene family in Arabidopsis thaliana. Genes & Development 22(10):1331–1336

Liu RX, Jia HT, Cao XL, Huang J, Li F, Tao YS, Qiu FZ, Zheng YL, Zhang ZX, 2012. Fine mapping and candidate gene prediction of a pleiotropic quantitative trait locus for yield-related trait in *Zea mays*. PLoS ONE 7(11): 1–12

Lu GH, Tang JH, Yan JB, Ma XQ, Li JS, Chen SJ, Ma JC, Liu ZX, E LZ, Zhang YR, Dai JR, 2006. Quantitative trait loci mapping of maize yield and its components under different water treatments at flowering time. Journal of Integrative Plant Biology 48(10): 1233-1243

Lu MY, Li XH, Shang AL, Wang YM, Xi ZY, 2011. Characterization of a set of chromosome single-segment substitution lines derived from two sequenced elite maize inbred lines. Maydica 56(4): 399-407

Messmer R, Fracheboud Y, Bänziger M, Vargas M, Stamp P, Ribaut JM, 2009. Drought stress and tropical maize: QTL-by-environment interactions and stability of QTLs across environments for yield components and secondary traits. Theor Appl Genet 119(5): 913-930

Millar N, 2001. Biology statistics made simple using Excel. School Science Review 83: 23-34

Neuffer MG, Coe EH, Wessler SR. Mutants of Maize. Cold spring harbor laboratory press, New York

Neuffer MG, England D, 1995. Induced mutations with confirmed locations. Maize Genetics Coop Newsl 69: 43-46

Neuffer MG, Sheridan W, Bendbow E, 1978. Rescue of lethal defective kernel mutants by genetic manipulation. Maize Genetics Coop Newsl 52: 84-88

Peng B, Li YX, Wang Y, Liu C, Liu ZZ, Tan WW, Zhang Y, Wang D, Shi YS, Sun BC, Song YC, Wang TY, Li Y, 2011. QTL analysis for yield components and kernel-related traits in maize across multi-environments. Theor Appl Genet 122(7): 1305-1320

Peng B, Wang Y, Li YX, Liu C, Liu ZZ, Wang D, Tan WW, Zhang Y, Sun BC, Shi YS, Song YC, Wang TY, Li Y, 2010. QTL analysis for yield components and kernel-related traits in maize under different water regimes. Acta Agron Sin 36(11): 1832-1842

Schnable PS, Ware D, Fulton RS, Stein JC, Wei F, Pasternak S, Liang C, Zhang J, Fulton L, Graves TA, Minx P, Reily AD, Courtney L et al, 2009. The B73 maize genome: complexity, diversity, and dynamics. Science 326(5956): 1112-1115

Schön CC, Melchinger AE, Boppenmaier J, BrunklausJung E, Herrmann RG, Seitzer JF, 1994. RFLP mapping in maize: quantitative trait loci affecting testcross performance of elite European flint lines. Crop Sci 34(2): 378-389

Sen TZ, Harper LC, Schaeffer ML, Andorf CM, Seigfried TE, Campbell DA, Lawrence CJ, 2010. Choosing a genome browser for a model organism database: surveying the maize community. Database the Journal of Biological Databases & Curation, 2010(1): 207-214

Soderlund C, Descour A, Kudrna D, Bomhoff M, Boyd L, Currie J, Angelova A, Collura K, Wissotski M, Ashley E, Morrow D, Fernandes J, Walbot V, Yu Y, 2009. Sequencing, mapping, and analysis of 27, 455 maize full-length cDNAs. PLoS Genet 5(11): e1000740

Song XJ, Huang W, Shi M, Zhu MZ, Lin HX, 2007. A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. Nat Genet 39: 623–630

Stuber CW, Lincoln SE, Wolff DW, Helentjaris T, Lander ES, 1992. Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. Genetics 132(3): 823-839

Su ZQ, Hao CY, Wang LF, Dong YC, Zhang XY, 2011. Identification and development of a functional marker of *TaGW2* associated with grain weight in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 122(1): 211-223

Van Ooijen JW, 2011. Multipoint maximum likelihood mapping in a full-sib family of an outbreeding species. Genetics research 93(05): 343-349

Veldboom LR, Lee M, 1994. Molecular-marker-facilitated studies of morphological traits in maize. II: Determination of QTLs for grain yield and yield components. Theor Appl Genet 89(4): 451-458

Veldboom LR, Lee M, 1996. Genetic mapping of quantitative trait loci in maize in stress and nonstress environments: I. Grain yield and yield components. Crop Sci 36(5): 1310-1319

Wang BT, Zhang SH, Xi ZY, 2012. QTL mapping for ear length based on chromosome single segment substitution lines of 87-1 zong3 in maize. Journal of Maize Sciences 20(3): 9-14

Wang S, Basten CJ, Zeng ZB, 2007. Windows QTL cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC

Xia T, Li N, Dumenil J, Li J, Kamenski A, Bevan MW, Gao F, Li YH, 2013. The ubiquitin receptor DA1 interacts with the E3 ubiquitin ligase DA2 to regulate seed and organ size in Arabidopsis. The Plant Cell 25(9): 3347–3359

Xiang DQ, Cao HH, Cao YG, Yang JP, Huang LJ, Wang SC, Dai JR, 2001. Construction of a genetic map and location of quantitative trait loci for yield component traits in maize by SSR markers. Acta Genetica Sinica 28(8): 778-784

Xiang DQ, Huang LJ, Cao YG, Dai JR, 2001. A preliminary study on genetic effect of maize yield component traits based on major gene and polygene mixed inheritance. Acta Agriculturae Boreali-Sinica 16(3): 1-5

Xiang DQ, Huang LJ, Dai JR, 1999. Progress in studies on maize yield QTL and the genetic basis of heterosis. Journal of China Agricultural University, S:1-7

Yan JB, Tang H, Huang YQ, Zheng YL, Li JS, 2006. Quantitative trait loci mapping and epistatic analysis for grain yield and yield components using molecular markers with an elite maize hybrid. Euphytica 149(1): 121-131

Yang JP, Rong TZ, 1999. Improvement of RFLP-marker-facilitated studies of QTLs in maize. Journal of Maize Sciences 7(1): 18-24

Yang JP, Rong TZ, Xiang DQ, Tang HT, Huang LJ, Dai JR, 2005. QTL mapping of quantitative traits in maize. Acta Agron Sin 31(2): 188-196

Yang ZB, Bai ZY, Li XL, Wang P, Wu QX, Yang L, Li LQ, Li XJ, 2012. SNP identification and allelic-specific PCR markers development for TaGW2, a gene linked to wheat kernel weight. Theor Appl Genet 125(5):1057-1068

Zhang GD, Wang XP, Wang B, Tian YC, Li M, Nie YX, Peng QC, Wang ZL, 2013. Fine mapping a major QTL for kernel number per row under different phosphorus regimes in maize (*Zea mays* L.). Theor Appl Genet 126(6): 1545-1553

Zhang K, Shi X, Zhao XF, Ding D, Tang JH, Niu JX, 2015. Investigation of miR396 and growth-regulating factor regulatory network in maize grain filling. Acta Physiologiae Plantarum 37(2):1-12

Zhang W, Sun P, He Q, Shu F, Wang J, Deng H, 2013. Fine mapping of *GS2*, a dominant gene for big grain rice. The Crop Journal 1(2): 160–165

Zhou GF, Zhu QL, Yang GL, Huang J, Cheng SY, Yue B, Zhang ZX, 2015. qEL7.2 is a pleiotropic QTL for kernel number per row, ear length and ear weight in maize (*Zea mays* L.). Euphytica 203(2): 429-436