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Genetic analysis of QTLs for lysine content in four maize DH populations

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Abstract

Background Low levels of the essential amino acid lysine in maize endosperm is considered to be a major problem regarding the nutritional quality of food and feed. Increasing the lysine content of maize is important to improve the quality of food and feed nutrition. Although the genetic basis of quality protein maize (QPM) has been studied, the further exploration of the quantitative trait loci (QTL) underlying lysine content variation still needs more attention.

Results Eight maize inbred lines with increased lysine content were used to construct four double haploid (DH) populations for identification of QTLs related to lysine content. The lysine content in the four DH populations exhibited continuous and normal distribution. A total of 12 QTLs were identified in a range of 4.42–12.66% in term of individual phenotypic variation explained (PVE) which suggested the quantitative control of lysine content in maize. Five main genes involved in maize lysine biosynthesis pathways in the QTL regions were identified in this study.

Conclusions The information presented will allow the exploration of candidate genes regulating lysine biosynthesis pathways and be useful for marker-assisted selection and gene pyramiding in high-lysine maize breeding programs.

Keywords Maize, Double haploid population, Lysine content, QPM, Genetic analysis

Introduction

As one of the main crops worldwide, maize (*Zea mays* L.) serves as an important source of nutrition [1–3]. However, the poor lysine content of maize greatly limits this essential amino acid for human and livestock [3–6]. Therefore, the lysine contents in maize endosperm are considered to be one of the most important traits for determining the nutritional quality of food and feed [5].

In order to improve breeding for balanced amino acid composition of maize kernels, many research efforts has been expended on identify the genes controlling amino acid content in the maize kernel and a large number of mutants related to maize endosperm have been found [3, 7]. The *opaque-2* (*o2*) mutation has about a 50% reduction in zeins and nearly doubles the lysine content of maize endosperm compared to normal genotypes [3, 8–10]. However, the soft and starchy endosperm associated with *o2* lead to

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brittleness and insect susceptibility, both in storage and in the field, thereby decreasing the value of the grain [11–13]. The alleles controlling the soft and starchy texture of *o2* endosperm, are designated as *opaque2* modifiers, this modified *o2* maize mutant is known as "Quality Protein Maize" or QPM [5, 14]. The QPM has been used in breeding programs to develop semi-vitreous and vitreous phenotypes with high lysine content and solve the problems related to the *o2* mutant [15]. However, the development and widespread use of QPM is limited because of the genetically complex germplasm and the technical complexity of the multiple loci [5]. Hence, the discovery of additional advantageous genes and enhancing our comprehension of the fundamental amino acid biosynthesis processes are crucial for developing quality protein maize varieties with higher lysine content [7, 16].

In previous studies, many QTLs related to maize lysine content or QPM traits have been identified. For instance, five significant QTLs for *o2* modifiers were identified in $F_{2:3}$ individuals from a cross between two isogenic QPM inbreds and were shown to influence the lysine content using functional and genomic SSR markers [17]. Holding et al. found seven major QTLs associated with *o2* endosperm modification from developed QPM lines and that genic regions on chromosomes 5, 7 and 9 might be major hubs of *o2* modifiers [11, 15]. Wang and Larkins identified four significant loci that account for about 46% of the phenotypic variance and coincident with the genes involved in free amino acids biosynthetic pathways [18]. Deng et al. identified four QTLs for lysine content in three RIL populations and one QTL were further validated using molecular approaches [7]. These QTLs aid in understanding the genetic basis of lysine quantity and quality, and facilitate genetic improvement of QPM in breeding programs.

The genetic analysis tends to be affected greatly due to the unique feature of diverse mapping populations [19]. Double haploid (DH) segregating populations have become the ideal materials to assist in different basic research studies in rice [20], oilseed rape [21, 22], maize [19, 23, 24], wheat [25, 26], barley [27]. It is helpful to discover the important genes and QTLs for multiple traits not merely because shortens the breeding process but also provides pure lines by filtering out residual heterozygosity [19, 24, 28–32]. Meanwhile, by conditioning linked markers in the test, the sensitivity of the test statistic to the position of individual QTLs is increased, and the precision of QTL mapping can be improved with the development of sequencing technology [19, 33–38]. Thus, the objective of the present study was to identify effective QTLs and analyze genetic basis for lysine content in four DH populations using SNP markers, and pinpoint the main genes which involved in lysine biosynthesis pathways in the QTL regions and hoped to provide insights into the genetic basis of lysine biosynthesis in maize kernels and may facilitate marker-based breeding for high lysine maize.

Materials and methods

Plant materials and lysine content measurement

Eight high quality parents, which exhibited significant differences in kernel quality traits, were from Maize Yufeng Biotechnology LLC and selected as elite inbred lines used for construction of four DH populations. The populations were planted at Liaoning province (LN, 40°`82′N, 123°56′E) with three replication blocks in 2021. The details about this field experiment were described previously [39, 40].

Near infrared reflectance (NIR) spectrometer (DA 7250, Perten Instruments Inc., Sweden) was used to estimate the lysine content in maize kernels. Each sample was scanned three times to obtain an averaged value. The phenotypic variation of lysine content was analyzed by using R software 4.0.1 with the "aov" function (ANOVA). The BLUP values were used for phenotypic description statistics and QTL analysis. These details were described previously [39, 40].

Genotyping and construction of genetic linkage map

The genotype of all lines was performed by using the GenoBaits Maize 2 K marker panel (Mol Breeding Biotechnology Co., Ltd., Shijiazhuang, China), containing 10,378 SNP markers [41]. The details about the quality control, data conversion and construction of genetic linkage maps have been described previously [39, 40].

QTL detection

The QTL analysis was conducted by using composite interval mapping (CIM) method implemented in Windows QTL Cartographer 2.5 [42]. The threshold empirical logarithm of the odds (LOD) was determined at a significance level of $p<0.05$ [43]. The details about the QTL detection have been described previously [39, 40].

Determination of key candidate genes

The genes related to lysine synthesis and metabolism pathways were listed from the relevant publications. The physical positions of these genes on the chromosomes were found on maizeGDB database (https://Chinese.maizegdb.org/). Finally, the key candidate genes were determined by comparison of the physical positions and QTL intervals in this study [18].

Results

Phenotypic variation in kernel lysine content

Four DH populations (AF109, AF116, AF129 and AF170) were developed by using eight inbred lines with lysine content ranging from 0.18 to 0.44% (Table 1). These populations contained 248, 190, 316 and 265 lines, respectively. The observed lysine content (0.12–0.50%) among individuals of the DH populations showed a continuous and normal distribution (Fig. 1).

Table 1 The phenotypic performance of eight parents and variance of lysine content in the four DH populations

Trait [a]	Populations							
	AF109		AF116		AF129		AF170	
Parents								
means ± SD (%)	KB319003 (female parent)	0.21 ± 0.03	KB519007 (female parent)	0.34 ± 0.04	KB320005 (female parent)	0.25 ± 0.04	JinQingWL2 (female parent)	0.18 ± 0.01
	AJ317001 (male parent)	0.38 ± 0.02	KB717001 (male parent)	0.43 ± 0.03	KB120001 (male parent)	0.37 ± 0.03	KB319004 (male parent)	0.44 ± 0.02
pvalue[b]	<0.0001****		<0.0002***		<0.0001****		<0.0001****	
DHs								
Size	248		190		316		265	
means ± SD (%)	0.32 ± 0.07		0.32 ± 0.07		0.33 ± 0.06		0.27 ± 0.08	
Range (%)	0.17–0.49		0.18–0.49		0.18–0.49		0.12–0.50	

[a] lysine content; [b] *P* value based on a t-test evaluating two parental lines. *** $p < 0.001$, **** $p < 0.0001$

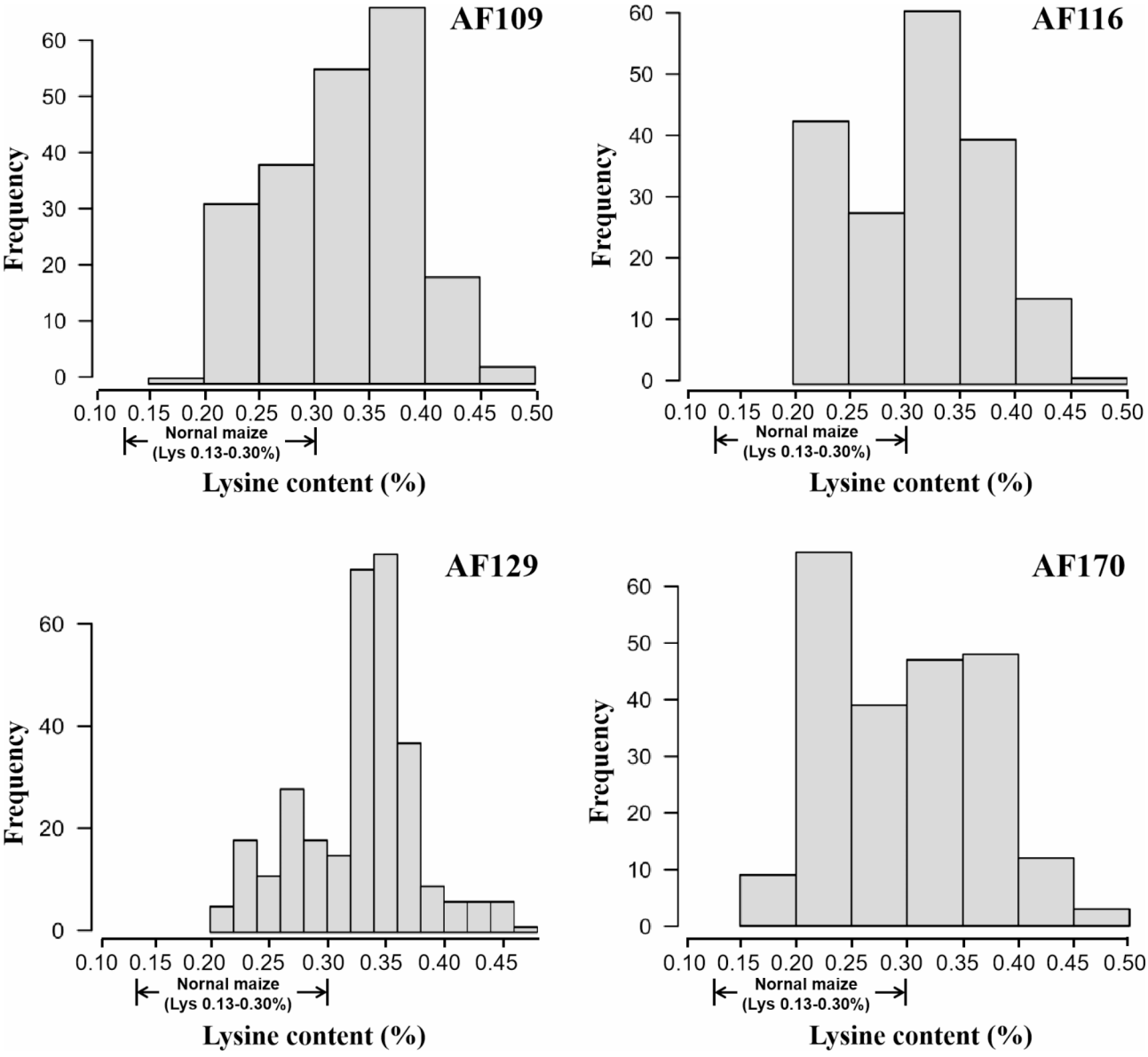

Fig. 1 Frequency distributions of lysine content in AF109, AF116, AF129 and AF170 populations. In normal maize endosperm, the range of lysine content is 0.130–0.300% [44, 45]

Table 2 Individual QTL for lysine content in the four DH populations

Populations	QTL	Chr.[a]	*P*-Peak (Mb)_V4[b]	*P*-Range (Mb)_V4[c]	LOD	PVE%[d]	Add.[e]	Parent[f]+	PVE(%) -ALL[g]
AF109	*qLYS-1-1*	3	69.67	59.07–97.50	3.994	4.42	-0.041	KB319003	11.94
	qLYS-1-2	4	44.01	37.64–65.21	3.867	4.67	0.017	AJ317001	
	qLYS-1-3	9	130.80	129.70-137.80	4.034	4.98	0.021	AJ317001	
AF116	*qLYS-2-1*	3	161.43	161.43–169.00	3.892	4.46	-0.022	KB519007	8.70
	qLYS-2-2	9	132.53	130.80-149.39	4.661	5.60	0.031	KB717001	
AF129	*qLYS-3-1*	1	267.92	267.37-271.96	4.226	5.57	0.083	KB320005	26.06
	qLYS-3-2	2	214.01	210.90-219.60	4.552	5.29	-0.014	KB120001	
	qLYS-3-3	6	131.71	117.49-141.72	6.731	12.66	0.016	KB320005	
AF170	*qLYS-4-1*	1	150.61	143.21-159.07	3.529	7.57	0.031	JinQingWL2	24.03
	qLYS-4-2	5	12.70	12.70-13.55	4.111	8.65	0.020	JinQingWL2	
	qLYS-4-3	9	111.07	93.07-125.24	4.990	6.92	0.028	JinQingWL2	
	qLYS-4-4	10	14.80	13.61–17.92	3.333	5.23	-0.020	KB319004	

[a] Chromosome; [b] Physical position of QTL based on the B73 reference sequence (V4); [c] Physical position range of QTL based on the B73 reference sequence (V4); [d] Percentage of the phenotypic variation explained by the additive effect of QTL; [e] Additive effect of QTL; [f] which parental allele increased lysine content; [g] Percentage of the phenotypic variation explained by the additive effect of all QTL

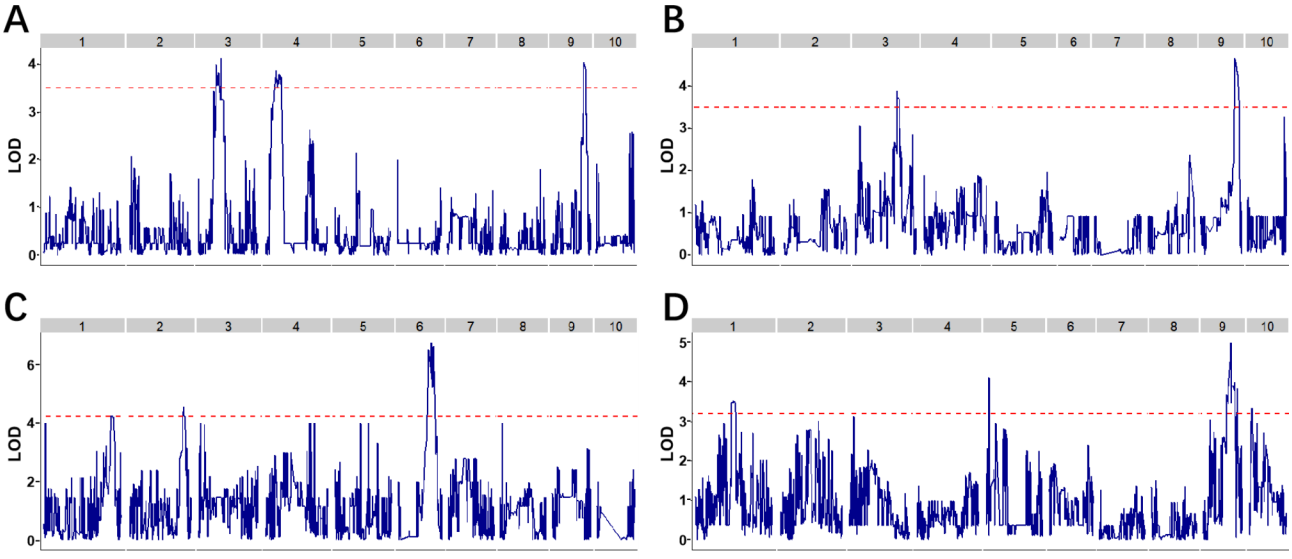

Fig. 2 The distribution of QTLs across the entire genome in the four DH populations. **A-D** designated AF109, AF116, AF129 and AF170 population, respectively

Genotyping and genetic linkage map

A total of 8,377 homozygous polymorphic SNPs were identified among the all DH lines in four populations by using GenoBaits Maize 2 K marker panel containing 10,378 SNP markers with MAF < 0.1 or missing rate > 0.6. Based on the reference parental polymorphic loci, four high density linkage maps were constructed for AF109, AF116, AF129 and AF170, respectively. These linkage maps consisted of 4269 bin markers and covered the ten maize chromosomes with an average distance of 0.79 cM between adjacent markers.

QTLs analysis of lysine content in four DH populations

QTL mapping for the four DH populations and CIM analyses were performed (Table 2). In total, 12 QTLs associated with lysine content were detected in the four DH populations at a LOD value of 3.0 after 1000 permutations. These QTLs were distributed among twelve genomic regions on chromosomes 1 through 6, 9 and 10 (Fig. 2). The confidence intervals for these QTLs spanned physical distances ranging from 0.85 to 32.17 Mb, with an average of 15.91 Mb. The lysine variation in these DH populations that could be explained by all of the detected QTLs was between 8.70% (AF116) and 26.06% (AF129), with each QTL ranging from 4.42 to 12.66% (*qLYS-3-3*). The largest QTL *qLYS-3-3* was located on chromosome 6. *qLYS-3-3* explained the greatest proportion of phenotypic variation indicating that it was the major QTL controlling lysine content in population AF129. The KB320005 allele at this locus had an additive effect of 1.6% for increased lysine content. The second larger QTLs *qLYS-4-1*, *qLYS-4-2* and *qLYS-4-3* were located on

chromosome 1, 5 and 9 and explained 6.92–8.65% of the phenotypic variation. Besides, they were all from population AF170 and had the alleles from JinQingWL2 contributed to increased lysine content.

Genetic overlap of QTLs in the four DH populations

To better understand the genetic basis of lysine content in maize kernel, the QTL overlaps among the populations were analyzed (Fig. 3). The QTL with overlapping support intervals were considered common QTL for lysine content. The results showed that only one overlap (7.00 Mb) between *qLYS-1-3* and *qLYS-2-2* was found on chromosome 9 among the four DH populations. Furthermore, by comparing the intervals with that of other publications [7, 11, 15, 17, 18], there were only 8.70 Mb and 7.57 Mb overlaps with the QTLs in a $F_{2:3}$ progeny derived from high free amino acids (FAA) parents Oh545*o2* and Oh51A*o2* [18].

Discussion

The quality assessment of genetic linkage maps

A series of parameters including molecular markers may affect the mapping of QTLs [46]. Because of the most frequent variations in the genome, the applications of SNP markers in QTL mapping studies have increased the pace and precision of plant genetic analysis and provide a high map resolution [37, 38, 47–49]. In our study, *qLYS-4-2* spanned the smallest physical interval of only 0.85 Mb, five QTLs (*qLYS-1-3*, *qLYS-2-1*, *qLYS-3-1*, *qLYS-3-2* and *qLYS-4-4*) spanned a physical interval less than 10 Mb and six QTLs (*qLYS-1-1*, *qLYS-1-2*, *qLYS-2-2*, *qLYS-3-3*, *qLYS-4-1* and *qLYS-4-3*) spanned relatively larger physical intervals (15.86–38.43 Mb), which were still less than 40 Mb. Thus, the resolution in this study is considerably improved because of the large number of markers and the appropriate population type [46].

Genetic analysis of lysine content in DH populations

The results of the phenotypic and genetic detection showed that there was a wide phenotypic variation for lysine content (0.12–0.50%) in the four DH populations. Among the identified QTLs, only two QTLs (*qLYS-1-3* and *qLYS-2-2*) spanned a 7.00 Mb physical interval on chromosome 9. Percentage of the phenotypic variations of 11 QTLs were less than 10%, except the PVE of *qLYS-3-3* was 12.66%. These results suggested that the genetic component of lysine content was controlled by many small effect QTLs. Moreover, by comparing with other studies, only two QTLs showed less than 10 Mb physical interval overlap with the results from a $F_{2:3}$ progeny derived from high FAA parents Oh545*o2* and Oh51A*o2* [18], which demonstrated the complexity of lysine content regulation in diverse genetic backgrounds. It was shown that, *qLYS-2-1* in AF116 shared 8.70 Mb with the QTL between flanking markers bmc1904-bmc1452, where *qLYS-3-2* in AF129 shared 7.57 Mb with the QTL between flanking markers bmc1537-bmc2248, which had the alleles contributed by Oh545*o2* responsible for high FAA level. Therefore, *qLYS-2-1* might be significantly associated with FAA content in maize kernel. The other

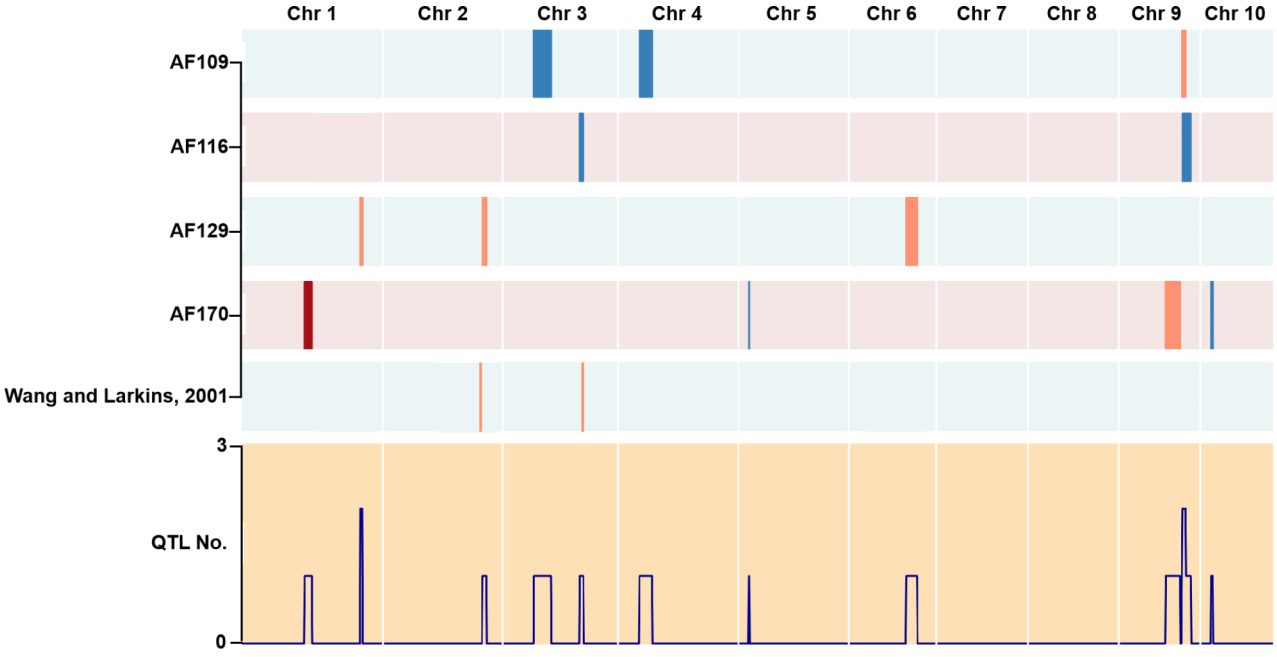

Fig. 3 Overlaps of QTLs for lysine biosynthesis among the DH populations and all types of other populations. The present and reported populations were labeled on the left and the number of identified QTLs was below

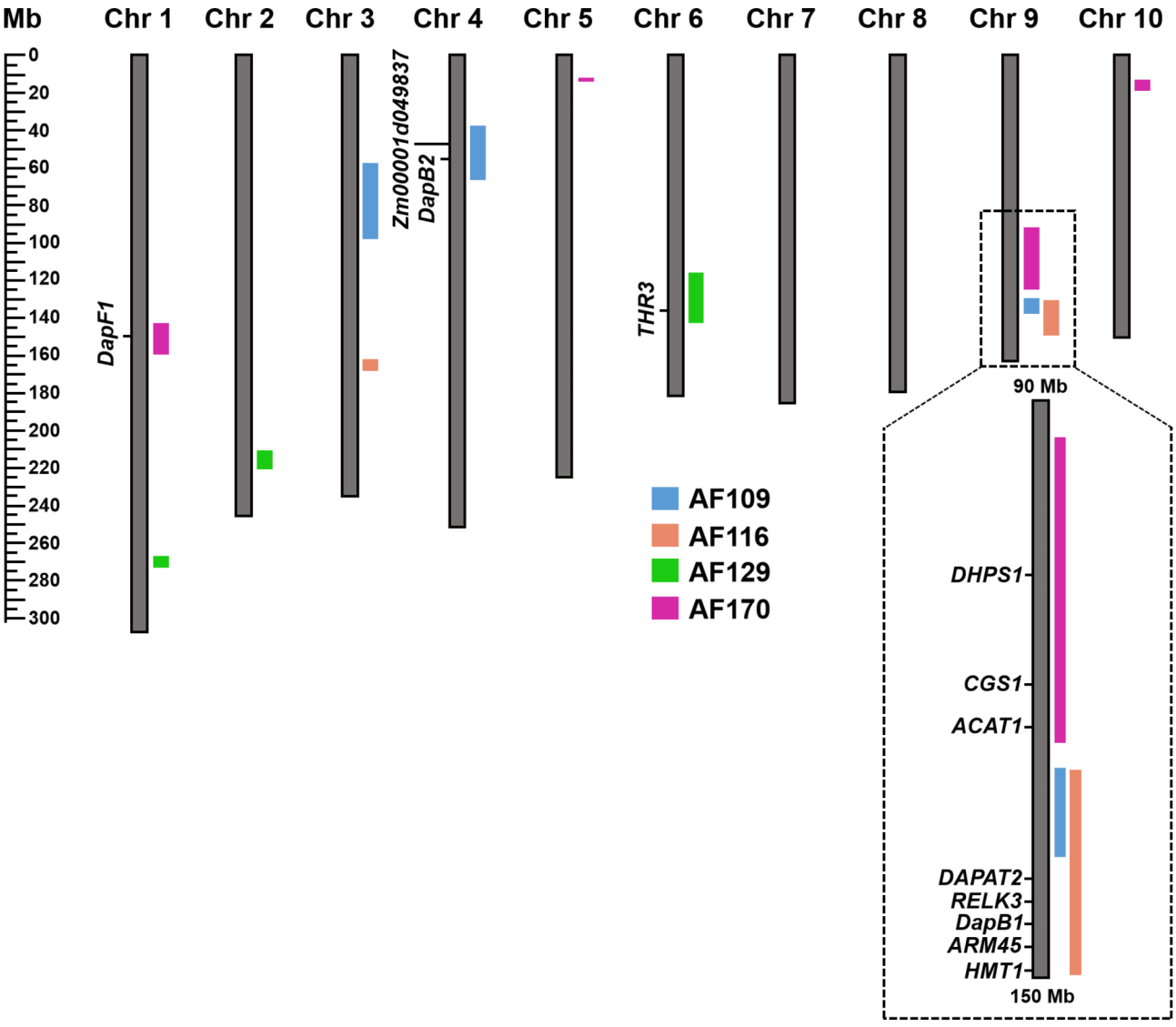

Fig. 4 The key candidate genes related to lysine biosynthesis pathway in present QTLs intervals. The QTLs identified in four DH populations are represented as vertical rectangles of different colors next to each chromosome. The left labels denote known genes that co-localized with the QTLs

QTLs in this study displayed few overlaps with regions associated for lysine content or related traits in multiple former studies [7, 11, 15, 17]. It was inferred that although some genetic loci may have a common effect on lysine content, there are still QTLs unique to different populations. The above analysis demonstrated that ten QTLs are newly discovered in this study with the exception of *qLYS-2-1* and *qLYS-3-2*, and merit further downstream research such as their application in marker-assisted selection (MAS) in breeding.

Candidate genes relevant to lysine content in maize genetics and breeding

In lysine biosynthesis there are two distinct pathways [6]. One is via α-aminoadipate which exists in fungi and *Euglena* [50]. The other is the diaminopimelate pathway which exists in bacteria, plants, and archaea [51]. Dihydrodipicolinate synthase (DHDPS) is the core enzyme in the diaminopimelate pathway and has primary roles in regulating the level of lysine accumulation in plant cells [52, 53]. In our study, the gene *DHPS1* (*Zm00001d046898*) encoding dihydrodipicolinate synthase1 was found on chromosome 9 and might be solely participated in lysine biosynthesis pathway network during maize seed development [6] (Fig. 4). Diaminopimelate epimerase (DapF) is one of the crucial enzymes involved in lysine biosynthesis, where it converts l, l-diaminopimelate (l, l-DAP) into d, l-DAP in the diaminopimelate pathway [6, 54–56]. The *DapF1* (*Zm00001d030677*) identified in our study in QTL *qLYS-4-1* might be an important gene involved in lysine biosynthesis. Dihydrodipicolinate reductase (DapB) catalyses

the second reaction in the diaminopimelate pathway of lysine biosynthesis [6, 57–60]. In this study, we identified two DapB genes, *DapB1* (*Zm00001d047935*) and *DapB2* (*Zm00001d049956*) on chromosome 9 and 4, respectively, which might be responsible for the key enzymes in the diaminopimelate- and lysine-synthesis pathways that reduces dihydrodipicolinate to tetrahydrodipicolinate. Aiaminopimelic acid (DAP) is a central intermediate that regulate the lysine biosynthesis in DAP-pathway [61]. In Arabidopsis, the LL-diaminopimelate aminotransferase was found directly regulate DAP synthesis bypassing the DapD-, DapC- and DapE catalyzed steps [6, 61]. It was indicating that the gene *diaminopimelate aminotransferase2* (*DAPAT2*) (*Zm00001d047695*) identified on chromosome 9 in AF116 DH population might have a unique role in maize lysine biosynthesis.

Conclusion

In this study, four DH populations were constructed for genetic analysis of maize lysine content and were normally distributed. One major and eleven minor effect QTLs were identified based on the genetic linkage map with LOD threshold of 3.00 and accounted for 4.42–12.66% of lysine content variation. It suggested that the genetic component of lysine content was controlled by many small effect QTLs. Ten of the QTLs have never been reported in any previous studies. Additionally, five main genes which are involved in lysine biosynthesis pathways were located near the QTL regions. The QTLs identified in the present study supply valuable information for future research and will be highly useful for exploration of candidate genes associated with lysine content and QPM germplasm.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-024-10754-9.

Supplementary Material 1

Supplementary Material 2

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Author contributions
B.L. and X.Z. conceived the study. H.G. designed the experiments. L.Z. and J.W. performed the experiments. Z.C. and J.L. analyzed the results. X.Z. wrote the manuscript. H.W. and L.C. provided scientific suggestions and revised the manuscript. All authors reviewed the manuscript.

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Data availability
Sequence data that support the findings of this study have been deposited in figshare repository. https://figshare.com/articles/dataset/Phenotype_and_genotype_of_Lys_in_four_populations/25709877.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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