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# Genetic Dissection of Isoleucine and Leucine Contents in the Embryo and Maternal Plant of Rapeseed Meal Under Different Environments

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**Abstract:** The genetic basis controlling the content of two essential amino acids (isoleucine and leucine) in rapeseed meal was investigated through a replicated trial of the two $BC_1F_1$ populations from a two-way backcross between 202 TN DH population strains and their parents ('Tapidor' and 'Ningyou7'). Given the impact of rapeseed embryos and maternal plants on seed qualities, a multi-genetic-system QTL mapping method was employed, incorporating both genetic main effects and environmental interaction effects. The results demonstrated the presence of nine QTLs associated with isoleucine and leucine content in the A1, A4, A5, A7, A9, and C2 linkage groups. These included six QTLs controlling isoleucine content and three QTLs controlling leucine content, which collectively explained 55.49% and 56.06% of the phenotypic variation, respectively. Of these, four QTLs were identified as the main QTL, which collectively explained over 10% of the phenotypic variation. All of the identified QTLs exhibited a highly significant additive and dominant effects on seed embryos. Additionally, one of the QTLs demonstrated had a particularly significant additive effect derived from the maternal genome. QTLs controlling isoleucine and leucine were identified in the A1, A4, and C2 linkage groups. Moreover, two QTL clusters influencing these essential amino acid contents were identified in the A4 and C2 linkage groups, situated between molecular markers HS-K02-2 and HBR094 and between EM18ME6-220 and NA12C03, respectively.

**Keywords:** rapeseed meal; isoleucine content; leucine content; embryo; maternal plant; genetic main effect; QTL and environmental interaction effect



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## 1. Introduction

In addition to its status as a significant oil crop, rapeseed (*Brassica napus* L.) also serves as a vital source of plant protein worldwide, making a substantial contribution to global edible oil supplies and agricultural output [1]. The use of rapeseed meal as a source of feed protein is of significant importance due to its amino acid composition, which is closely aligned with the nutritional quality of the feed [2]. Isoleucine and leucine are essential amino acids for the majority of animals [3]. The contents of isoleucine and leucine in rapeseed meal belong to complex quantitative traits and have a complex genetic basis. The genes controlling the expression of isoleucine and leucine quality traits in rapeseed can be expressed in the embryonic nuclear genome, the maternal plant nuclear genome, or simultaneously in both nuclear genomes [4,5]. It has been demonstrated that the amino acid content of crop seeds is regulated by multiple genes and is susceptible to environmental

influences. A variety of QTL (Quantitative Trait Locus) mapping methods and models have been employed to identify the QTL regulating amino acid content in rice [6–8], wheat [9], soybean [10], cotton [11], and rapeseed [12,13]. However, there is a paucity of information regarding the QTL mapping of isoleucine and leucine content in rapeseed. Based on the mixed linear model, Yang et al. [14] proposed that the full QTL model and mapping strategy could be used to dissect the polygenic genetic system of complex traits into QTL main effects and their interactions with environmental factors in various experimental populations. The corresponding computer software (QTLNetwork-CL-2.0-Seed) was developed with the objective of detecting the QTLs in the two genetic systems of the seed embryo and maternal plant simultaneously. It has been successfully applied to the QTL mapping of two genetic systems for quality traits of crops [7,8,11,12,15]. However, there are no reports on QTL mapping of two genetic systems for the isoleucine and leucine content in rapeseed.

The objective of this study is to identify the QTLs that regulate the isoleucine and leucine content in rapeseed. This will be achieved by mapping the QTLs across different years using the QTL mapping method of multiple genetic systems, including environmental interaction effects and corresponding mapping software. The mapping will be conducted on the embryo and maternal plant genomes. The distribution differences of these QTLs with embryo additive main effect, embryo dominant main effect, and maternal additive main effect, as well as their environmental interaction effects will be elucidated. This could provide a scientific basis for marker-assisted selection (MAS) of related traits and cloning of main genes in the future.

## 2. Materials and Methods

### *2.1. Materials*

The 202 double haploid (DH) strains derived from the cross between 'Tapidor' and 'Ningyou7' (TN) were constructed by Huazhong Agricultural University [16]. The male parent, 'Ningyou7' is a Chinese semi-winter variety with the characteristics of increased sulfur glycoside, erucic acid, and yield. The female parent, 'Tapidor' is a European winter variety that displays lower glucosinolate and erucic acid levels, along with a moderate yield. The two parental strains (Tapidor and Ningyou7) and 202 DH populations were kindly provided by Professor Meng Jinling's laboratory at Huazhong Agricultural University.

### *2.2. Field Experiments*

In the initial seasons of 2011 and 2012, two parental strains and 202 DH populations were planted on the experimental farm of Zhejiang University. The seeds were sown in early October of each year and transplanted to a field with a spacing of 25 cm × 25 cm in a randomized block design with two replications after 40 days. A two-way backcross between the DH population and their parents was conducted to obtain the QTL mapping populations $BC_1F_1$ 1 (DHs × Tapidor) and $BC_1F_1$ 2 (DHs × Ningyou7), of which 191 and 177 $BC_1F_1$ groups were obtained in the spring of 2012 and 2013, respectively. At the maturity stage, the seeds of the parents and the two-way backcross population were harvested, and the contents of isoleucine and leucine in the rapeseed were measured.

### *2.3. Trait Measurement*

The spectroscopic collection and amino acid content determination of rapeseed meal were conducted by using a Near Infrared Scanning Monochromator (Model 5000 NIRS Systems Inc., Silver Spring, MD, USA) and the WinISI II software (v1.5, FOSS NIRSystems, Silver Springs, MD, USA) to determine the isoleucine content and leucine content in rapeseed with about 3 g/sample in a small ring cup of 36 mm (inner diameter) under a room temperature of 25 °C [12,17].

### *2.4. Statistical Analysis*

SAS 9.1 (Statistics Analysis System) software (SAS Institute, Cary, NC, USA) was used for descriptive statistics and phenotypic analysis including mean value, standard deviation, maximum value, minimum value, skewness, peak value, and correlations among the isoleucine and leucine contents.

### *2.5. Linkage Genetic Map and QTL Mapping*

The QTL mapping genetic map used in the experiment was constructed by the National Key Laboratory of Crop Genetic Improvement in Huazhong Agricultural University with a total of 786 molecular markers, including RFLP (Restriction Fragment Length Polymorphism), MS-RFLP (Microsatellite Restriction Fragment Length Polymorphism), SSR (Simple Sequence Repeat), STS (Sequence Tagged Site), SNP (Single Nucleotide Polymorphism), SSCP (Single-Strand Conformation Polymorphism), AFLP (Amplified Fragment Length Polymorphism), and CAPS (Cleaved Amplified Polymorphic Sequences). The total genome size was 2117.2 centimorgans (cM), which covered all 19 chromosomes of *Brassica napus* (AACC, 2n = 38), and the average distance between two markers was 2.7 cM [18]. QTL analyses with environmental interaction effects were carried out on the isoleucine and leucine content in rapeseed by using the QTL Network-CL-2.0-Seed software and the mixed-model-based composite interval mapping (MCIM) method with a 10 cM window size and a 1 cM walking speed [12,14,19]. QTLs were named according to the McCouch standard nomenclature [20].

## 3. Results

### *3.1. Phenotypic Variation of Isoleucine and Leucine Content*

The results demonstrated that the isoleucine and leucine contents of 'Tapidor' in the two-year test were markedly elevated in comparison to those of 'Ningyou 7' except for the difference in isoleucine content between the parents in 2013 (Table 1). In the backcross population, the isoleucine and leucine contents of the two $BC_1F_1$ in 2012 and 2013 were found to be lower than those of their parents. The contents of the two essential amino acids in the two $BC_1F_1$ populations exhibited a single-peak continuous variation in the phenotypic distribution maps of different environments (Figure 1). The absolute values of skewness and kurtosis were lower than 1, indicating that these two traits exhibited a normal distribution and were therefore quantitative traits controlled by multiple genes. Furthermore, the phenotypic variation of the backcross offspring demonstrated a one-way separation from the superparent, and the performance of the same trait exhibited variability under diverse environmental conditions. This suggested that the contents of isoleucine and leucine in rapeseed were only minimally influenced by environmental factors.

**Table 1.** The quality traits of *Brassica napus* L. including Ile and Leu of parents and two backcross populations.

| Growth | Traits | Parent | | $BC_1F_1$ (DH × Tapidor) | | | | | | $BC_2F_1$ (DH × Ningyou7) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Year | | Tapidor | Ningyou7 | Means | SD | Minimum | Maximum | Skewness | Kurtosis | Means | SD | Minimum | Maximum | Skewness | Kurtosis |
| 2012 | Ile | 1.602 | 1.430 ** | 1.427 | 0.084 | 1.220 | 1.689 | 0.189 | 0.120 | 1.386 | 0.081 | 1.180 | 1.684 | 0.548 | 0.878 |
| | Leu | 2.912 | 2.525 ** | 2.563 | 0.125 | 2.271 | 2.935 | 0.224 | 0.164 | 2.493 | 0.137 | 2.121 | 2.919 | 0.381 | 0.492 |
| 2013 | Ile | 1.409 | 1.402 | 1.272 | 0.083 | 1.043 | 1.499 | 0.072 | −0.172 | 1.223 | 0.071 | 1.079 | 1.428 | 0.356 | 0.092 |
| | Leu | 2.660 | 2.462 ** | 2.351 | 0.127 | 2.015 | 2.694 | −0.106 | −0.086 | 2.254 | 0.115 | 1.998 | 2.581 | 0.199 | −0.026 |

** indicate significant differences of target traits between two parents at $p = 0.01$ according to the *t*-test. Ile and Leu are the abbreviations for isoleucine and leucine, respectively.

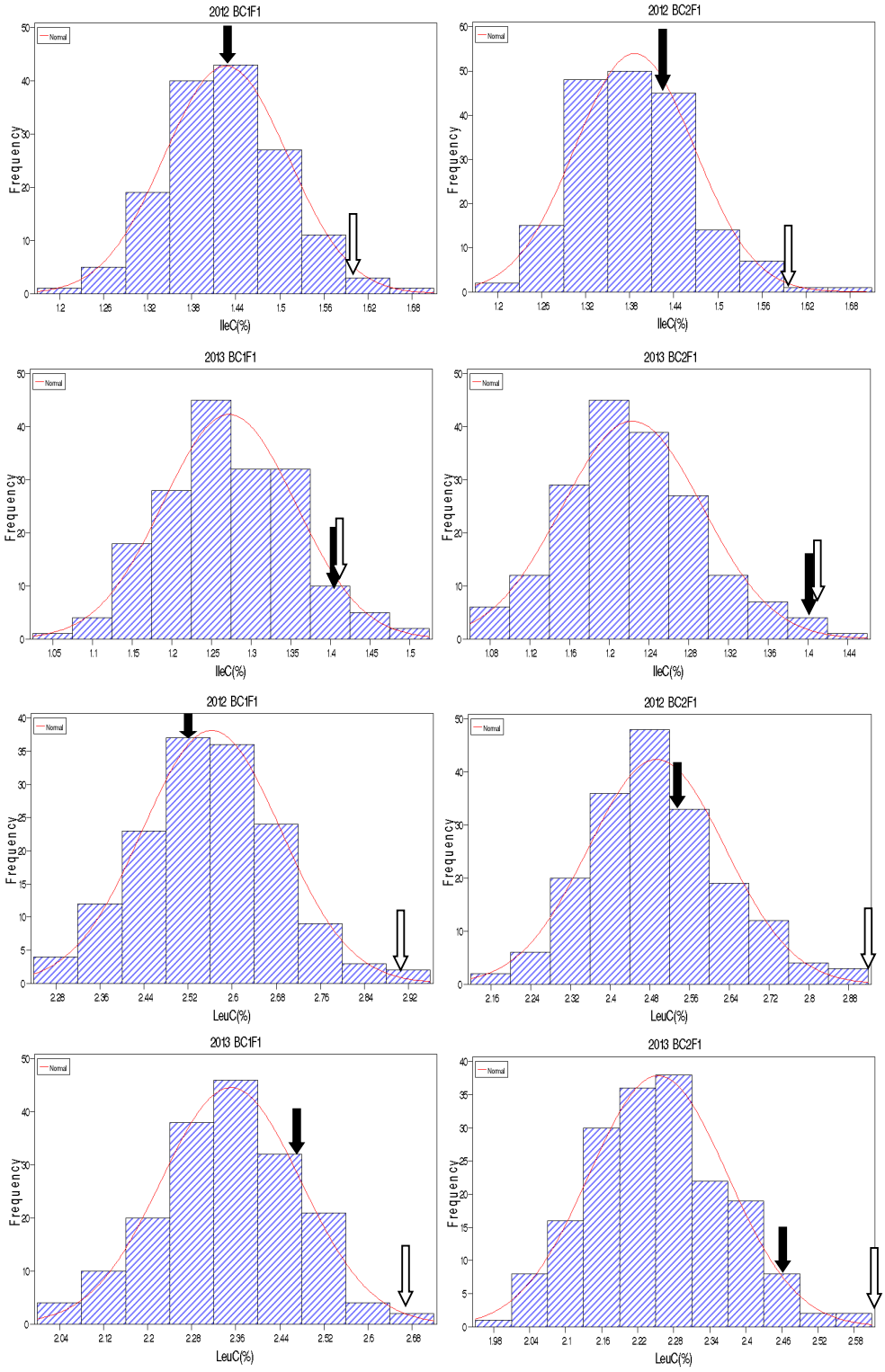


**Figure 1.** Frequency distributions of Ile and Leu for $BC_1F_1$ (DHs × Tapidor) and $BC_2F_1$ (DHs × Ningyou7) in 2012 and 2013. White and black arrows indicate the average values of the parents Tapidor and Ningyou7, respectively.

### *3.2. Correlation Analysis of Isoleucine and Leucine Content*

A significant positive correlation was demonstrated between isoleucine content and leucine content in rapeseed, with a correlation coefficient of 0.963. This indicates a highly evident correlation between the two traits. The results indicated that the isoleucine

content and leucine contents of rapeseed could be simultaneously enhanced through quality breeding.

### *3.3. QTL Mapping Analysis*

This study identified nine QTLs that regulate isoleucine content and leucine content, exhibiting significant embryo additive and dominant effects on embryo development. Among these, *qIleC-4-2* exhibited particularly pronounced additive effects from the maternal plant. Among the detected QTLs, six QTLs controlling isoleucine and three QTLs controlling leucine could account for 55.49% and 56.06% of the phenotypic variation, respectively. Furthermore, *qIleC-4-2*, *qIleC-7-4*, *qLeuC-1-1*, and *qLeuC-4-2* were the principal QTLs controlling related traits, with the capacity to explain more than 10% of the phenotypic variation (Table 2 and Figure 2). The findings of this study suggested that the expression of QTLs associated with isoleucine content and leucine content in rapeseed was relatively stable, predominantly influenced by genetic factors and less susceptible to environmental fluctuations.

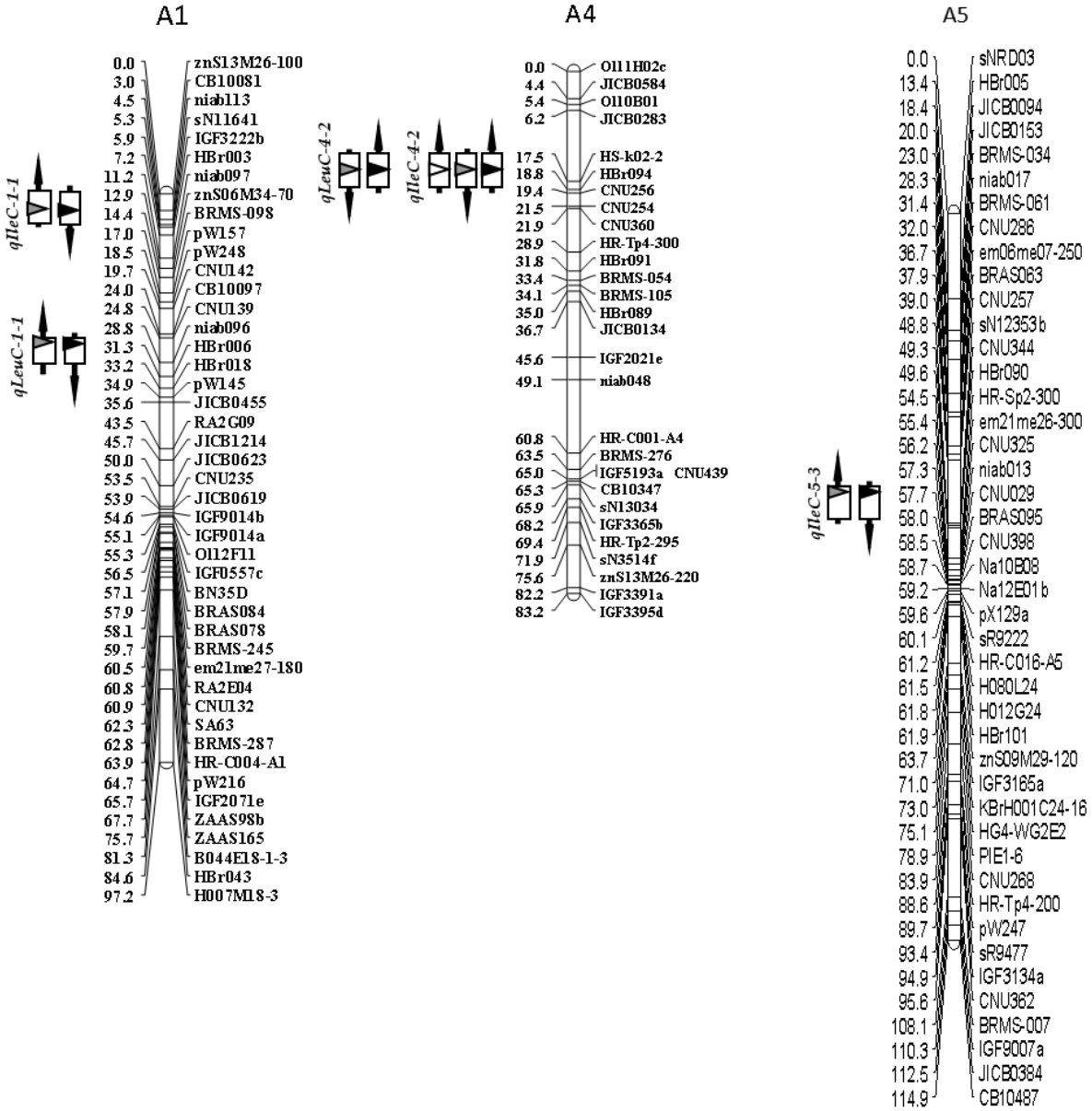


**Figure 2.** *Cont.*

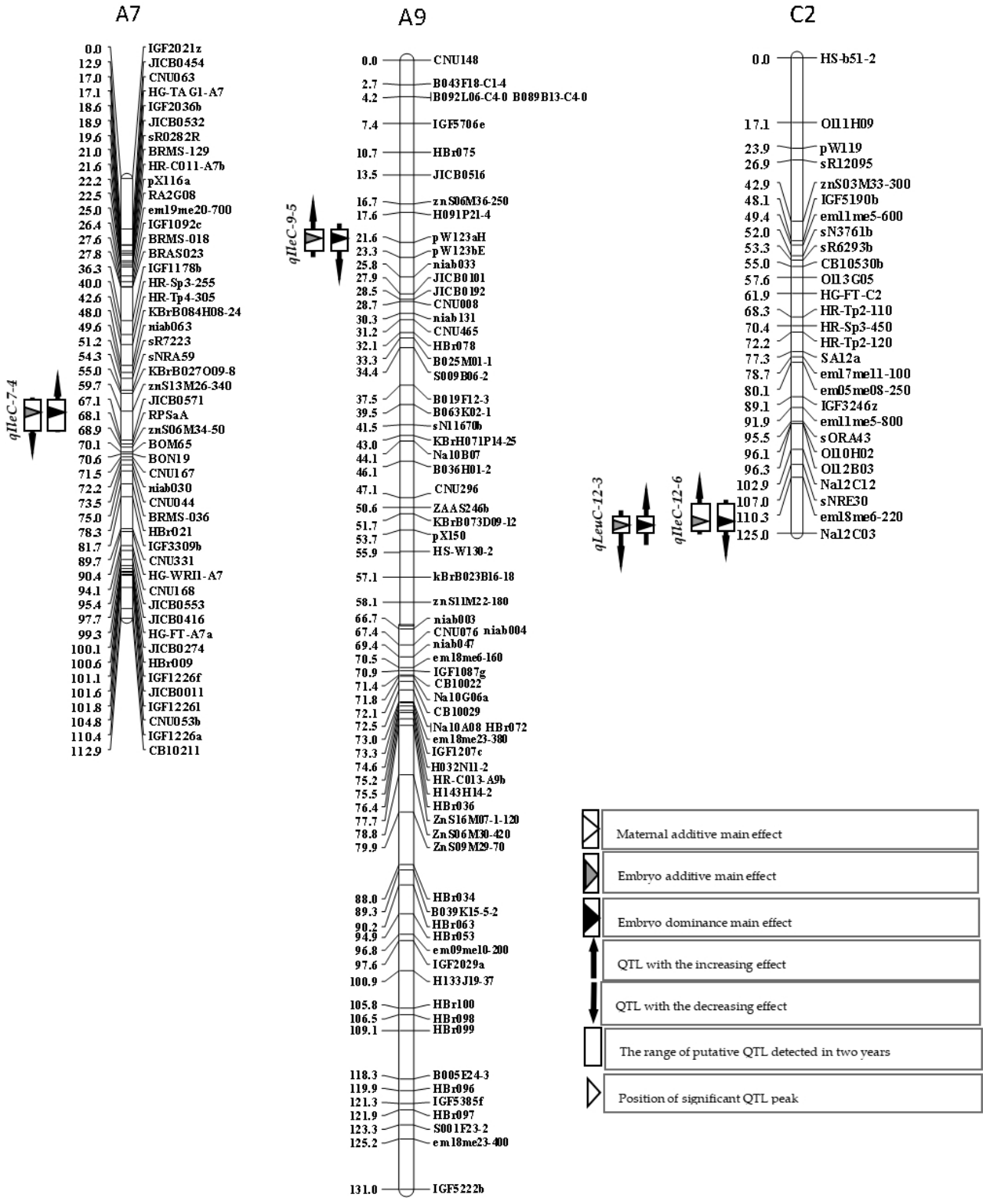


**Figure 2.** Mapping of the QTLs for controlling Ile and Leu in $BC_1F_1$ (DHs × $P_1$) and $BC_2F_1$ (DHs × $P_2$) of DHs derived from the cross Tapidor × Ningyou7.

**Table 2.** QTL locations and effects for Ile and Leu contents in $BC_1F_1$ (DHs × Tapidor) and $BC_2F_1$ (DHs × Ningyou7) of DHs under the different environments.

| QTL | Linkage Group | Marker Interval | Position | Range | $R^2$ | $A_e$ | $D_e$ | $A_m$ | $A_eE_1$ | $D_eE_1$ | $A_mE_1$ | $A_eE_2$ | $D_eE_2$ | $A_mE_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *qIleC-1-1* | A1 | BRMS-098-PW157 | 14.4 | 12.9–15.4 | 3.26 | 0.2788 ** | −0.262 ** | −0.0004 | 0.0026 | −0.0026 | 0.0043 | 0.0001 | −0.0001 | −0.0043 |
| *qIleC-4-2* | A4 | HS-K02-2-HBR094 | 18.5 | 17.5–19.4 | 19.43 | −0.6764 ** | 0.6444 ** | 0.0104 ** | 0.0002 | −0.0002 | −0.0002 | 0 | 0 | 0.0002 |
| *qIleC-5-3* | A5 | CNU029-BRAS095 | 57.7 | 57.3–58.0 | 4.19 | 0.3125 ** | −0.301 ** | 0.0034 | 0.0001 | −0.0001 | 0.0021 | 0.0002 | −0.0002 | −0.002 |
| *qIleC-7-4* | A7 | RPSAA-ZNS06M34-50 | 68.1 | 67.1–68.9 | 18.37 | −0.6531 ** | 0.6301 ** | 0.0007 | 0.0002 | −0.0002 | −0.0035 | 0 | 0 | 0.0034 |
| *qIleC-9-5* | A9 | PW123AH-PW123BE | 21.6 | 19.6–23.3 | 4.51 | 0.3253 ** | −0.3112 ** | −0.0017 | 0.0003 | −0.0003 | 0 | 0.0001 | −0.0001 | 0 |
| *qIleC-12-6* | C2 | EM18ME6-220-NA12C03 | 116.3 | 108.0–124.3 | 5.73 | 0.3653 ** | −0.3519 ** | 0.0028 | 0.0039 | −0.0038 | 0.0026 | 0.0001 | −0.0001 | −0.0027 |
| *qLeuC-1-1* | A1 | HBR006-HBR018 | 31.3 | 29.8–32.3 | 35.67 | 1.2792 ** | −1.2431 ** | 0.0019 | 0.0004 | −0.0004 | 0.0003 | −0.0002 | 0.0002 | −0.0003 |
| *qLeuC-4-2* | A4 | HS-K02-2-HBR094 | 18.5 | 15.2–19.4 | 13.16 | −0.7823 ** | 0.7504 ** | −0.0003 | 0.0001 | −0.0001 | −0.0002 | −0.0014 | 0.0014 | 0.0002 |
| *qLeuC-12-3* | C2 | EM18ME6-220-NA12C03 | 118.3 | 111.3–124.3 | 7.23 | −0.5526 ** | 0.5782 ** | 0.0023 | 0.0076 | −0.0075 | 0.0072 | −0.0002 | 0.0002 | −0.0073 |

Note: ** indicate significance at the levels of 1%. $A_e$, embryo additive main effect; $D_e$, embryo dominance main effect; $A_m$, maternal additive main effect; $A_eE_1$, embryo additive interaction effect in environment 1; $D_eE_1$, embryo dominance interaction effect in environment 1; $A_mE_1$, maternal additive interaction effect in environment 1; $A_eE_2$, embryo additive interaction effect in environment 2; $D_eE_2$, embryo dominance interaction effect in environment 2; $A_mE_2$, maternal additive interaction effect in environment 2.

### 3.4. QTL Controlling Isoleucine Content

Six QTLs controlling isoleucine content in rapeseed were detected, which were distributed in the A1, A4, A5, A7, A9, and C2 linkage groups of the *Brassica napus* genome. These were designated as *qIleC-1-1*, *qIleC-4-2*, *qIleC-5-3*, *qIleC-7-4*, *qIleC-9-5*, and *qIleC-12-6*, respectively (Table 2 and Figure 2). These QTLs could account for 55.49% of the phenotypic variation in isoleucine content in rapeseed. Among these, *qIleC-4-2* (located between the molecular markers HS-K02-2 and HBR094 in the A4 linkage group) and *qIleC-7-4* (located between RPSAA and ZNS06M34-50 in the A7 linkage group) were identified as the primary QTLs responsible for 19.43% and 18.37% of the phenotypic variation, respectively. Nevertheless, *qIleC-1-1* ($R^2$ = 3.26%), *qIleC-5-3* ($R^2$ = 4.19%), *qIleC-9-5* ($R^2$ = 4.51%), and *qIleC-12-6* ($R^2$ = 5.73%) were identified as minor QTLs that exert control over isoleucine content. Among these, *qIleC-1-1*, *qIleC-5-3*, *qIleC-9-5*, and *qIleC-12-6* were all identified with a highly significant positive embryo additive main effect and embryo dominant main effect, indicating a reducing effect. Additionally, *qIleC-4-2* and *qIleC-7-4* were also identified with a significant additive main effect and dominant effect on the embryo, although their action directions were opposite. Notably, *qIleC-4-2* was detected with a very significant positive additive effect on the maternal plant. Furthermore, no significant interaction effect between maternal plant and environment was identified in the QTL controlling isoleucine content in rapeseed. This suggested that isoleucine content was predominantly influenced by embryo additives and dominant main effects, with no evident correlation with environmental conditions.

### 3.5. QTL Controlling Leucine Content

The three QTLs controlling leucine content, identified in the A1, A4, and C2 linkage groups, were designated as *qLeuC-1-1*, *qLeuC-4-2*, and *qLeuC-12-3*, respectively (Table 2 and Figure 2). Of these, *qLeuC-1-1* and *qLeuC-4-2*, which were located at corresponding marker intervals between HBR006 and HBR018 in the A1 linkage group and between HS-K02-2 and HBR09 in the A4 linkage group, might be the primary QTLs controlling the leucine content of rapeseed. This was due to their respective contributions of 35.67% and 13.16% to the phenotypic variation, respectively. *qLeuC-12-3* ($R^2$ = 7.23%), situated between the EM18ME6-220~NA12C03 intervals in the A1 linkage group, was a relatively minor QTL. The collective contribution of the identified QTLs to the phenotypic variation was found to be 56.06%, with the majority of this variation being attributed to embryo additive and dominant effects. No discernible maternal effect was observed. Among the identified QTLs, *qLeuC-1-1* exhibited a synergistic embryo additive main effect and a reducing embryo dominant main effect. Similarly, *qLeuC-4-2* and *qLeuC-12-3* demonstrated notable embryo additive main effects and embryo dominant main effects, albeit with opposing directions of QTL action compared to *qLeuC-1-1*. Furthermore, no significant maternal plant additive main effect or environmental interaction effect was observed in the QTLs controlling leucine content, indicating that the expression of QTL for leucine content in rapeseed was minimally influenced by the maternal plant additive main effect and exhibited only a weak response to environmental factors.

### 3.6. QTL Co-Location Analysis

The results presented in Table 2 and Figure 2 demonstrate that some QTLs were identified at the same chromosomal locus. In the A4 linkage group, *qIleC-4-2* and *qLeuC-4-2* were found to be co-located between molecular markers HS-K02-2 and HBR094, exhibiting with a positive embryo additive main effect and a negative embryo dominant main effect. Additionally, QTLs associated with isoleucine content and leucine content (*qIleC-12-6* and *qLeuC-12-3*) were also co-located between the interval of molecular marker EM18ME6-220 and NA12C03 in the C2 linkage group. However, the direction of the embryo additive main effect and the embryo dominant main effect of *IleC-12-6* were opposite to that of *qLeuC-12-3*. This indicated the potential existence of genes regulating the expression of isoleucine and leucine at these two loci. The results of the QTL co-mapping analysis indicated the potential

for close linkage between different QTLs or for a single QTL to exert multiple effects at these loci or regions.

## 4. Discussion

The provision of balanced nutrients in animal feed makes essential amino acids a significant nutrient factor in rapeseed meal [21,22]. Given that rapeseed meal is subsequent to the extraction of rapeseed oil, the amino acid content of the meal is determined by the genetic composition of the seeds from which it is derived. A review of the literature reveals that the amino acid contents of rapeseed meal were quantitative traits with a complex genetic basis that were affected by both genetic and environmental factors [4,5,23]. The complex quantitative trait genes could be decomposed into a single Mendelian genetic gene [24,25], and the QTL controlling the quality quantitative traits in rapeseed could be mapped by some quantitative trait QTL mapping methods and software with the molecular marker linkage map [26–30]. Zhu and Weir [19] devised and employed a mixed linear model for QTL mapping which was not constrained by the limitations of traditional mapping methods and software. The QTL-mapped model was capable of not only analyzing the additive, dominant, and epistatic effects of QTL but also of simultaneously analyzing the interaction effects between QTL and the environment. However, given that rapeseed is a dicotyledonous crop, the observed variation in amino acid content in rapeseed might be attributable to the influence of different genetic systems, including those associated with the seed embryo and maternal plant, as well as those related to genotypes × environmental interactions [12]. It was therefore necessary to consider the QTL main effect and QE effect of different genetic systems of the seed embryo and maternal plant when mapping the QTL of isoleucine and leucine content in rapeseed.

The two essential amino acid contents, specifically those of isoleucine and leucine, in rapeseed are important nutritional quality traits in rapeseed meal, with a complex genetic basis. As illustrated in Table 1 and Figure 1, the parental traits exhibited superparental segregation in the backcross offspring population, indicating a high degree of genetic polymorphism in isoleucine content and leucine content between the two parents. Furthermore, the two traits exhibited a normal distribution (Figure 1), confirming that isoleucine content and leucine content were quantitative traits regulated by multiple genes. This indicated that conventional single-gene mapping methods were unsuitable for these two traits. The newly developed QTL mapping method based on two sets of genetic systems from embryo and maternal plants and mapping software (QTLNetwork-CL-2.0-Seed), offered a novel approach to comprehensively understanding the genetic mechanisms of isoleucine content and leucine content in rapeseed [14]. In the present study, a total of nine QTLs controlling isoleucine content and three QTLs controlling leucine content were identified. All of these exhibited highly significant seed embryo additive main effects and dominant main effects. One QTL associated with isoleucine content exhibited a highly significant maternal additive main effect, yet no discernible QTL × environmental interaction effect was observed. It was therefore necessary to consider the influence of genetic information expressed from the embryonic and maternal plant genomes simultaneously when investigating the genetic mechanisms underlying isoleucine content and leucine content in rapeseed. Furthermore, four major QTLs ($R^2$ = 13.16–35.67%) and their closely linked molecular markers related to the isoleucine and leucine content were newly located. These could provide a reference value for QTL fine mapping and MAS of subsequent related traits. The correlation analysis conducted on leucine content and isoleucine content revealed a highly significant positive correlation between the two traits ($R$ = 0.963 **), indicating the presence of genes or QTLs on the rapeseed chromosome genome that might simultaneously regulate the synthesis of leucine and isoleucine. Additionally, QTLs regulating the isoleucine and leucine contents (*qLeuC-4-2*, *qIleC-4-2*, *qLeuC-12-3*, and *qIleC-12-6*) were identified within the two molecular marker intervals of A4 and C2 linkage groups, Furthermore, *qIleC-1-1* and *qLeuC-1-1* were both situated within the A1 linkage group. These QTLs might be in close linkage or have a one-factor pleiotropic effect. The phenomenon of QTL clustering had been observed in the

study of additional quality traits in rapeseed [12,31–33]. In particular, Wen et al. [12] also identified QTLs associated with histidine, glutamate, proline, alanine, and aspartate within the molecular marker interval HS-K02-2~HBR094. Furthermore, both highly significant embryo additive and embryo dominant effects were identified on *qIleC-4-2* and *qLeuC-4-2*, with the additive effect values exceeding those of the dominant effect values. *qIleC-4-2* and *qLeuC-4-2* were identified as the main QTLs controlling isoleucine content and leucine content, respectively. Among the identified QTLs, *qIleC-4-2* was showed with highly significant maternal additive effects, while no significant environmental interaction effects were observed for either *qIleC-4-2* or *qLeuC-4-2*. This suggested that the common QTL locus (HS-K02-2~HBR094) for *qIleC-4-2* and *qLeuC-4-2* might regulate leucine synthesis with greater stability, whereas the expression of isoleucine traits was susceptible to the maternal plant influence. Consequently, the co-located molecular markers HS-K02-2 and HBR094 could be employed in the subsequent MAS breeding to improve the isoleucine and leucine contents in rapeseed, thereby providing a reference for the cloning of the principal genes associated with these traits. A further benefit of the QTL mapping analysis of multiple genetic systems for isoleucine content and leucine content in rapeseed would be to research the effects of chromosome genomes of embryos and maternal plants under different environments on gene expression controlling the isoleucine content and leucine content. Additionally, the distribution and effects of related QTLs on rapeseed chromosome linkage groups could be better understood, providing new ideas and assistance for molecular design breeding of rapeseed quality traits in the future.

## 5. Conclusions

The present study identified twelve QTLs that regulate the contents of isoleucine and leucine in rapeseed. Furthermore, the effects of these QTLs, originating from the seed embryo and maternal plant nuclear genomes, and related QTLs × environmental interaction effects, were analyzed. These identified QTLs exhibited a highly significant additive main effect and dominant main effect from the embryonic genome. Additionally, a QTL related to the isoleucine content was detected, displaying a significant additive main effect from the maternal plant genome. No significant QTL × environmental interaction effects were detected. Two QTL were identified as co-localized in the A4 and C2 chromosome linkage groups, indicating that there were QTLs controlling the expression of isoleucine and leucine contents concurrently. The aforementioned results demonstrated that the expression of the two essential amino acids in rapeseed was influenced by the expression effect of the two different genetic systems in the embryo and maternal plant.

**Author Contributions:** Investigation, formal analysis, writing—original draft, J.X.; Validation, methodology, H.X.; Conceptualization, methodology, C.S.; validation, writing—review and editing, Y.Z.; Data curation, investigation, Writing—review and editing, project administration. Z.Z.; Conceptualization, methodology, supervision, J.W. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** The original contributions presented in this study are included in the article; further inquiries can be directed to the corresponding author.

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**Conflicts of Interest:** The authors declare that they have no conflicts of interest.

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