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Study of Grain Size in a Recombinant Inbred Line Population Derived from Yunpi 2 × Large-Grained Barley

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ABSTRACT This study utilized two parental lines, "Yunpi 2," a locally selected variety from Yunnan with notable differences in functional component content, and a large-grained barley variety with characteristic hooked awns, to construct an F5 generation recombinant inbred line (RIL) population ("Yunpi 2 × Large-Grained Barley"). Precise measurements of grain length, width, and thickness were conducted on 169 genotypes, including the two parental lines and 167 RILs, followed by genetic analysis. The objective was to select germplasm resources with superior grain traits, providing crucial materials for barley breeding and gene cloning. The results indicated significant differences in grain length between the two parental lines (difference value > 1), with most offspring grain lengths falling between the parental values but averaging lower than the parental mean. In contrast, differences in grain width and thickness were relatively small (difference value < 0.5), with the majority of offspring exhibiting widths and thicknesses greater than or equal to the larger parental value and averaging higher than the parental mean. This study demonstrates that grain size in barley RILs is closely related to parental grain size, with genetic traits significantly influenced by parental characteristics, providing important theoretical and practical guidance for barley variety improvement and genetic breeding.

***Keywords**: Barley; Inbred Lines; Grain Size; Genetics; Food*

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INTRODUCTION Barley (*Hordeum vulgare* L.), a member of the Poaceae family, is an annual grain crop characterized by a short growth period, strong stress resistance, and wide adaptability. Rich in dietary fiber, vitamins, minerals, and various bioactive components, barley serves as a nutritious food source and is widely cultivated globally (Schulte et al., 2009, Ahmed et al., 2025c). In the food sector, barley is a staple in many regions, particularly in high-altitude or challenging climates (Newton et al., 2011, Wang et al., 2019). In the feed industry, its nutritional richness and palatability make it a preferred feed ingredient for livestock and poultry (Blake et al., 2011, Ahmed et al., 2025a). Additionally, barley is a critical raw material for brewing beer, with its quality and yield directly impacting the beverage industry (Gupta et al., 2010, Fang et al., 2020).

Grain size is a crucial yield-related trait in barley, directly influencing single-plant yield and grain quality. Larger grains typically contain higher levels of starch, protein, and other nutrients, enhancing their performance in food processing and feed utilization (Walker et al., 2013, Shrestha et al., 2022). In brewing, grain size also affects malt yield and beer quality. Therefore, delving into the genetic mechanisms of barley grain size and developing larger-grained, higher-yielding, and superior-quality barley varieties through genetic improvement hold significant practical implications for global food security, livestock farming, and the brewing industry.

Recombinant inbred line (RIL) populations are valuable genetic resources for studying agronomic traits due to their relatively uniform genetic background, fixed genotypes, and reusability. These populations encompass the full genetic variation of the parental lines, facilitating the detection of numerous quantitative trait loci (QTL) and providing insights into the genetic basis of agronomic traits (Kang et al., 2020, Du et al., 2019). By evaluating phenotypic performance and conducting molecular marker analysis across different environments, the stability of QTL and their environmental interactions can be more accurately assessed, unveiling the genetic regulatory networks of agronomic traits (Yan et al., 2017, Shrestha et al., 2022, Jamshidi and Mohebbalipour, 2014).

In this study, we constructed a genetically stable F5 generation RIL population using "Yunpi 2" and large-grained barley as parental lines, measured grain length, width, and thickness, and conducted genetic analysis to identify superior germplasm resources for accelerating the breeding of high-yielding and high-quality barley varieties and providing valuable materials for elucidating the regulatory mechanisms of barley grain development and quality formation.

MATERIALS AND METHODS

Experimental materials

An F5 generation RIL population ("Yunpi 2 × Large-Grained Barley") consisting of 167 lines was constructed using "Yunpi 2" (R5201) and "Large-Grained Barley" (R5202) as parental lines. All the aforementioned experimental materials were planted at the Songming Experimental Base in Kunming City, Yunnan Province. The sowing date was November 1, 2024, and the seed harvest date was March 30, 2025. The seeds were sown using the strip sowing method in three replicated randomized block designs (Yang et al., 2025).

Experimental methods

Grain samples from 169 barley lines (including 2 parental lines and 167 RILs) were threshed, and 3 grains from each sample were randomly selected, their awns removed, and their length (mm), width (mm), and thickness (mm) measured using vernier calipers. Data were processed and statistically analyzed using EXCEL and SPSS 16.0 software, and frequency distribution histograms of grain length, width, and thickness in the RIL population were plotted (Peakall and Smouse, 2006, Jamshidi and Mohebbalipour, 2014).

RESULTS AND DISCUSSION

Grain Length

In this study, grain length showed substantial variation among the recombinant inbred lines (RILs), reflecting the complex genetic basis of this quantitative trait. The parental lines R5201 and R5202 exhibited mean grain lengths of 7.37 mm and 9.21 mm, respectively (Table 1, Figure 1). Within the RIL population, the mean grain length was 8.17 ± 0.39 mm. This intermediate mean suggests that the trait is inherited quantitatively and is influenced by alleles from both parents. Notably, only three RILs exhibited grain lengths below the lower parental value of 7.37 mm, with an average of 7.20 mm, suggesting rare instances of negative transgressive segregation. These lines might possess unique recombination of alleles resulting in trait suppression.

Most of the RILs, however, fell within the parental range, with a majority clustering between 7.37 mm and 9.21 mm and averaging 8.18 mm. This range-bound distribution indicates a general retention of parental trait values across the RILs. The pattern of grain length variation across the population approximated a normal distribution and demonstrated continuous variation with a wide amplitude, which is a hallmark of polygenic inheritance. This continuous variation aligns with the behavior of other complex traits governed by multiple genes, environmental interactions, and epistatic effects (Figure 1).

Grain Width

The parental lines also exhibited notable differences in grain width, with R5201 averaging 3.57 mm and R5202 showing a slightly smaller value of 3.31 mm (Table 1, Figure 2). The RIL population had a mean grain width of 3.69 ± 0.18 mm, slightly exceeding both parental means. Only four RILs had grain widths less than 3.31 mm, averaging 3.22 mm, demonstrating negative transgressive segregation similar to that observed in grain length. However, a substantial portion of the population (126 lines, or 75%) showed grain widths greater than the higher parental value (3.57 mm), averaging 3.77 mm. This distribution clearly indicates strong positive transgressive segregation.

The high frequency of RILs with values exceeding the parental range suggests the accumulation of complementary alleles from both parents that enhance grain width. These results imply that alleles from both R5201 and R5202 contribute positively to grain width, and through recombination, new superior allele combinations have emerged in the RILs. The grain width data also followed a continuous variation pattern with a broad amplitude, closely fitting a normal distribution curve (Figure 2). This again confirms the polygenic nature of this trait and highlights the effectiveness of the RIL population for mapping quantitative trait loci (QTLs) associated with grain width

Grain Thickness

The evaluation of grain thickness revealed further insights into the inheritance of grain shape traits. The parental lines R5201 and R5202 had mean grain thicknesses of 2.67 mm and 2.37 mm, respectively (Table 1, Figure 3). The RIL population exhibited a higher mean grain thickness of 2.72 ± 0.18 mm. Only four RILs displayed values below the lower parental limit, averaging 2.23 mm, while 57 RILs fell between the two parental values with an average of 2.55 mm. Interestingly, a majority of the lines—102 in total (61%)—exhibited values exceeding the higher parental mean, averaging 2.84 mm. This suggests strong positive transgressive segregation for grain thickness as well.

The wide variability observed in grain thickness, similar to grain length and width, underscores the complex polygenic control of this trait. The frequency distribution of grain thickness also approximated a normal curve and displayed a large amplitude of variation (Figure 3). This result is indicative of a multifactorial inheritance pattern and highlights the suitability of RIL populations for dissecting the genetic basis of complex traits like grain thickness.

Table 1: Descriptive statistical analysis of grain size in RILs population

Traits	Germplasm	Name and parameters	Values
Grain length	Parent	R5201	7.37
		R5202	9.21
	RILs population	Range	6.95 ~ 9.08
		Mean ± SD	8.17 ± 0.39
Grain width	Parent	R5201	3.57
		R5202	3.31
	RILs population	Range	3.10 ~ 4.15
		Mean ± SD	3.69 ± 0.18
Grain thickness	Parent	R5201	2.67
		R5202	2.37
	RILs population	Range	2.29 ~ 3.11
		Mean ± SD	2.72 ± 0.18

Genetic Complexity and Implications for Breeding

Grain size and shape in barley are outcomes of intricate interactions between genetic components and environmental conditions. Despite considerable research efforts, the comprehensive genetic control mechanisms underlying these traits remain partially understood. Numerous studies have aimed to unravel the QTLs responsible for grain size parameters. For instance, Walker et al. (2013) and Ahmed et al. (2025b) identified 232 QTLs associated with grain traits across three different environments, employing doubled haploid (DH) populations. These findings emphasize the high level of genetic complexity and environmental sensitivity associated with grain morphology.

In terms of specific loci, major QTLs for grain length have been reported on chromosomes 3H and 4H (Zhou et al., 2016; Bommisetty et al., 2020). Moreover, genome-wide association studies (GWAS) have further advanced our understanding, with Xu et al. (2018) identifying 29 QTLs related to grain size and weight across diverse barley genotypes. Despite these significant advancements in QTL mapping, none of these QTLs have yet been successfully cloned or definitively linked to specific functional genes directly controlling grain size. This underlines the complex nature of these traits and suggests that further fine mapping, functional validation, and genomic selection are required to achieve conclusive results.

Breeding strategies targeting grain morphology often focus on modifying grain shape to improve yield-related parameters. For example, selecting lines with wider grains and relatively shorter lengths has been proposed as an effective approach to increase thousand-grain weight (Kumar et al., 2020; Nadolska-Orczyk et al., 2017). Grain width and thickness are positively correlated with grain mass and are easier to enhance through conventional selection due to their higher heritability compared to grain length.

In the context of this study, the observation of positive transgressive segregation for both grain width and thickness indicates a promising opportunity for selecting elite lines with superior grain filling characteristics. The presence of lines with extreme values outside the parental range further suggests that favorable allele combinations were generated through recombination in the RIL population.

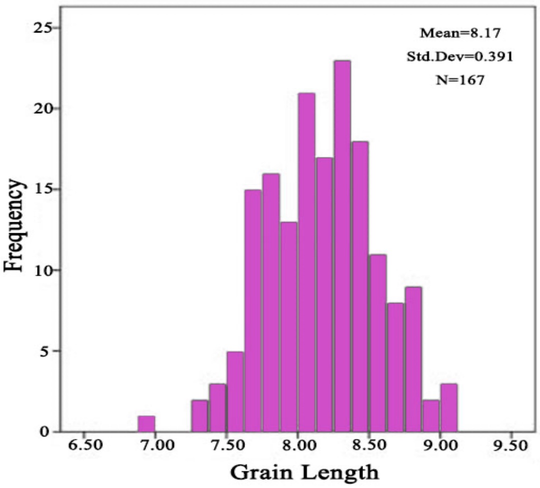

Figure 1: Frequency distribution histogram of grain length

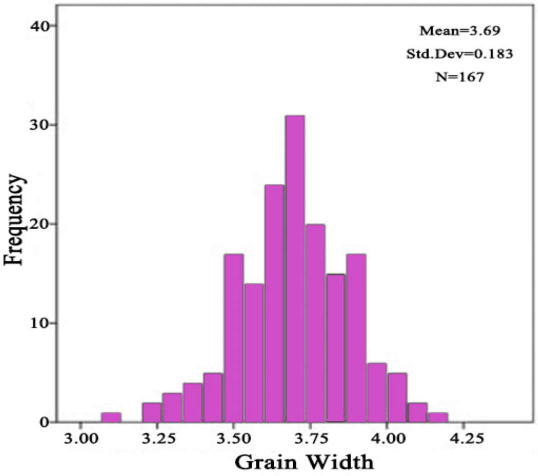

Figure 2: Frequency distribution histogram of grain width

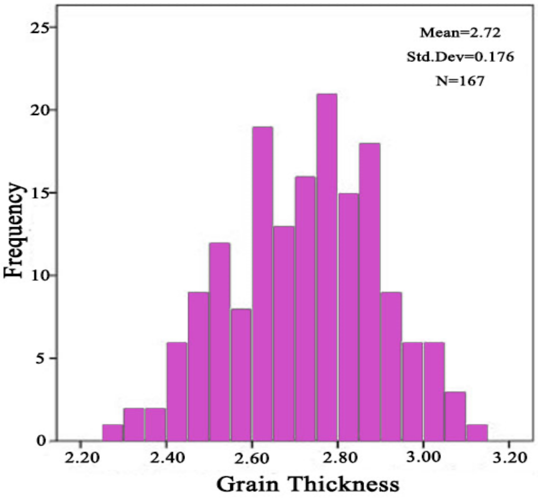

Figure 3: Frequency distribution histogram of grain thickness

Challenges and Future Directions

The study also highlights the inherent challenges in dissecting the genetic architecture of complex traits such as grain size. Traits like grain length, width, and thickness do not follow simple Mendelian inheritance but are controlled by numerous small-effect loci and are influenced by gene–gene and gene–environment interactions. This polygenic nature complicates traditional genetic analysis and breeding strategies. Furthermore, environmental factors such as temperature, moisture, and soil fertility can also exert significant effects on grain development, thus adding another layer of complexity.

Future research should aim to integrate high-throughput phenotyping technologies with next-generation sequencing and multi-environment trials to improve the resolution of QTL mapping. Advanced genomic tools such as CRISPR/Cas9-based gene editing and transcriptome analysis can also facilitate the functional validation of candidate genes underlying key traits. Moreover, incorporating genomic selection approaches into breeding programs could accelerate the improvement of grain morphology traits by predicting the breeding values of untested genotypes using molecular markers.

This study demonstrates that grain length, width, and thickness in barley RILs are quantitatively inherited traits with wide phenotypic variation and transgressive segregation, particularly in grain width and thickness. These traits are under complex genetic control and can be effectively exploited in breeding programs aimed at improving grain yield and quality. Continued efforts in molecular mapping, gene discovery, and advanced breeding techniques are essential to fully harness the genetic potential for grain size improvement in barley.

CONCLUSION

Grain size is a core factor influencing barley yield, with grain length, width, and thickness being key indicators for evaluating barley quality. Systematic and in-depth research on the genetic characteristics of grain size-related traits is crucial for enhancing barley yield and optimizing quality. Our analysis of barley grain traits revealed unique genetic patterns for different traits. Specifically, significant differences in grain length were observed between the parental lines (difference value > 1), with offspring grain lengths almost entirely falling within the parental range but averaging lower than the parental mean. In contrast, grain width and thickness showed relatively small differences between the parental lines (difference value < 0.5), with the majority of offspring exceeding the larger parental values and averaging higher than the parental means in these traits. This finding underscores the strong correlation between barley RIL grain size and parental grain size, highlighting the critical role of parental selection in breeding larger-grained barley varieties. Moreover, the RIL population used in this study offers advantages such as a clear genetic background, wide trait segregation, and relatively stable genotypes, providing high-quality materials for in-depth research on barley grain size genetics and contributing to the revelation of the genetic mysteries of barley grain size traits.

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