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Phenotyping advances for grain size associated traits in Wheat with emphasis on maximizing QTL discovery

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ABSTRACT

Grain size is a critical trait in wheat (*Triticum aestivum* L.) breeding, directly impacting yield, quality, and market value. Complex and highly influenced by genetic and environmental factors, grain size-associated traits such as grain length (GL), grain width (GW), grain area size (GAS), grain perimeter length (GPL), grain length-width ratio (GLWR), grain circularity (CS), and crease depth (CD) present significant challenges for precise phenotyping. This communication highlights advancements in high-throughput phenotyping methods, with a focus on tools like Smart Grain and recent innovations in artificial intelligence (AI) and deep learning that enable multi-trait measurement. High-resolution phenotyping not only enhances the precision of trait measurement but also maximizes the discovery of quantitative trait loci (QTLs) by capturing subtle genetic variations. The integration of AI-driven tools addresses the limitations of traditional methods by improving data processing speed and accuracy, thus facilitating a more comprehensive understanding of genotype-by-environment interactions. Emphasis is placed on how enhanced phenotyping techniques can be leveraged to identify minor but significant QTLs, particularly for complex traits like CD, which play a crucial role in nutrient transport and grain morphology. By integrating multi-dimensional trait data, modern phenotyping approaches support more robust QTL mapping, paving the way for improved wheat varieties with enhanced yield stability and adaptability.

KEY WORDS: *Wheat*; *Grain size*; *Phenotyping*; *Artificial intelligence*; *QTL mapping*

1. Introduction

Grain size is one of wheat's most significant agronomic traits (*Triticum aestivum* L.), playing a critical role in determining grain yield and market value. As wheat remains a global staple crop, feeding nearly 40% of the population, improving its productivity and quality is essential for meeting the rising demands of an expanding global population. Grain size traits, including grain length (GL), grain width (GW), grain area size (GAS), grain perimeter length (GPL), grain length-width ratio (GLWR), grain circularity (CS), and crease depth (CD), are pivotal components of grain morphology, influencing both yield potential and end-use quality. Breeding programs aiming to enhance wheat productivity often prioritize these traits due to their direct impact on economic value and functional characteristics. However, grain size traits are

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inherently complex, being influenced by multiple genes and their interactions with environmental factors. This complexity necessitates precise phenotyping and genetic characterization to fully understand their genetic basis and advance wheat improvement efforts.

QTL mapping has proven to be a powerful approach for identifying genomic regions associated with phenotypic variation in complex traits like grain size. By pinpointing these regions, researchers can identify candidate genes responsible for desirable characteristics, thus facilitating the development of superior wheat varieties. However, the accuracy of QTL mapping is heavily dependent on the quality and precision of phenotypic data. Poorly measured traits or limited data can lead to inconsistencies in QTL identification, diminishing the utility of marker-assisted selection (MAS) and genomic selection (GS). The complexity of grain size traits, coupled with their sensitivity to environmental conditions, underscores the need for advanced phenotyping technologies to achieve reliable genetic analysis and trait improvement.

Despite the importance of phenotyping, traditional methods for measuring grain size traits are fraught with limitations. These methods are often labor-intensive, time-consuming, and prone to human error, particularly when handling large populations or field-scale experiments. In addition to being ineffective, manual measurement of metrics like GL, GW, or CD is subjective, which produces inconsistent findings.

Furthermore, traditional phenotyping approaches are often constrained by their inability to simultaneously measure multiple traits at high precision, which limits their utility in multi-trait analyses. For example, while traits like grain length and area are commonly measured, critical parameters such as crease depth, which influences nutrient transport, grain filling, and milling efficiency, remain underexplored due to the lack of reliable tools for their quantification.

Recent advancements in high-throughput phenotyping technologies have begun to address these challenges. Automated systems like Smart Grain have emerged as powerful tools for analyzing grain size traits with high precision and scalability. These tools leverage image analysis algorithms to measure a range of parameters, including GL, GW, GAS, and GPL, in a fraction of the time required by manual methods. Smart Grain, for instance, allows researchers to phenotype large populations rapidly and consistently, facilitating multi-environment trials where high-resolution phenotypic data are crucial. By enabling more precise measurements, these technologies enhance the accuracy of QTL mapping and gene discovery, allowing researchers better to understand the genetic architecture of grain size traits.

The area has seen additional transformation with the integration of machine learning (ML) and artificial intelligence (AI) techniques into phenotyping operations. AI-driven tools, particularly those utilizing deep learning algorithms, can analyze complex datasets and automate the measurement of grain traits with unparalleled accuracy. Convolutional neural networks (CNNs), a subset of deep learning, have been applied to extract detailed phenotypic information from high-dimensional images, enabling the simultaneous analysis of traits such as GL, GW, GAS, and CD. These advancements not only improve the precision of phenotyping but also make it possible to analyze genotype-environment interactions, which are critical for understanding trait variability across different conditions.

A key focus in contemporary phenotyping is the integration of multiple grain size traits into a single framework. Multi-trait analyses are essential for maximizing QTL discovery, as they allow researchers to account for correlations between traits and identify genetic determinants that might be overlooked in single-trait studies. For example, traits like GL and GW often exhibit genetic correlations and analyzing them together can provide deeper insights into their underlying genetic basis. Similarly, incorporating parameters such as CD into phenotypic datasets can lead to the identification of new QTLs, thus broadening the scope of genetic improvement. Multi-trait approaches also enhance the robustness of breeding strategies, enabling the simultaneous selection of multiple desirable traits.

2. Grain size traits in wheat

Grain size associated traits comprise multiple parameters that collectively determine wheat yield and grain quality. Each of the following trait-parameter provides unique information about the physical characteristics of grain. Key traits include,

2.1 Grain length (GL): Grain length is one of the most important morphological traits influencing grain weight and end-use quality. Multiple studies have identified major QTLs associated with GL, such as TaGL3 and TaGS5, which play a role in cell elongation and division during grain development (Wang *et al*., 2015; Fan *et al*., 2019). Image-based phenotyping platforms, such as Smart Grain, have significantly improved GL measurement by providing high-resolution and automated analyses (Tanabata *et al*., 2012).

2.2 Grain width (GW): Grain width is another key component influencing kernel plumpness and milling quality. Studies indicate that GW is regulated by both pleiotropic and trait-specific QTLs, including TaCWI (cell wall invertase) genes that influence grain filling (Jiang *et al*., 2011). High-throughput imaging systems have enabled more precise GW measurements, improving the accuracy of genetic mapping (Du *et al*., 2016).

2.3 Grain area size (GAS): Grain area size is a composite trait derived from length and width measurements, providing an integrated parameter for grain morphology. GAS has been linked to major QTLs, such as qGAS.2D, and is strongly associated with grain yield (Zhang *et al*., 2016). Advanced phenotyping tools, such as Plant Screen, allow for large-scale GAS quantification in diverse populations (Fiorani and Schurr, 2013).

2.4 Grain perimeter length (GPL): Grain perimeter length serves as a descriptor of grain shape and can indirectly indicate kernel surface area and volume. Studies have highlighted its correlation with yield-related traits and its utility in discriminating between wheat varieties (Feng *et al*., 2018). Software like Smart Grain has been instrumental in generating accurate GPL measurements in breeding programs (Tanabata *et al*., 2012).

2.5 Grain length-width ratio (GLWR): The grain length-width ratio is a critical shape descriptor influencing market preference and milling efficiency. GLWR is under strong genetic control, with loci such as TaGLWR3-1 playing a prominent role in its regulation (Wu *et al*., 2019). High-throughput phenotyping systems allow for efficient GLWR measurement across diverse genotypes.

2.6 Grain circularity (CS): Grain circularity reflects kernel roundness, an essential trait for processing quality. Circular grains often have better milling efficiency and flour extraction rates. CS is now routinely measured using AI-powered tools, which analyze this trait alongside other size parameters (Ubbens and Stavness, 2017).

2.7 Distance between IS and CG (DS): The distance between the intersection of length and width (IS) and center of gravity (CG) provides novel insights into kernel symmetry and shape. This trait has emerged as an important indicator of grain uniformity, and its phenotyping is increasingly integrated into multi-trait analyses.

Crease depth (CD): Crease depth significantly affects grain hardness, milling yield, and susceptibility to fungal infections (Mabille and Abecassis, 2003; Sun *et al*., 2007; Kamaral *et al*., 2022). Despite its importance, CD phenotyping has been challenging due to limitations in traditional methods. Advances in 3D imaging and deep learning have enabled more precise measurements, paving the way for its inclusion in QTL interval mapping and genome wide-association studies (Ruan *et al*., 2020; Song *et al*., 2023).

3. Challenges in traditional phenotyping methods

Traditional phenotyping methods are labor-intensive, time-consuming, and often subject to operator bias. For example, manual measurement of traits like CD and CS is prone to inconsistencies, particularly in large-scale studies (Cobb *et al*., 2013). This underscores the need for advanced, high-throughput phenotyping tools. (Xu *et al*., 2017).

4. Advanced phenotyping approaches for grain size

High-Throughput Phenotyping (HTP): HTP systems leverage imaging technologies such as RGB cameras, near-infrared spectroscopy (NIR), and laser scanning to measure grain traits efficiently (Fiorani and Schurr, 2013). Platforms like LemnaTec and PlantScreen enable the rapid phenotyping of thousands of grains, significantly enhancing data collection.

Smart Grain imaging tool: Smart Grain software offers a high-throughput solution for phenotyping, with capabilities for capturing multiple traits simultaneously, which is essential for complex traits like grain size. By using high-resolution imaging and algorithmic processing, Smart Grain provides a comprehensive view of grain morphology that surpasses traditional methods (Tanabata *et al*., 2012). The software is particularly useful for traits such as GL, GW, and GAS, where accuracy and consistency are critical for QTL discovery.

However, Smart Grain also has limitations. Its effectiveness is reduced under conditions where grain morphology deviates from standard shapes, as the software may not accurately detect irregular grain boundaries. Additionally, Smart Grain's ability to incorporate environmental data is limited, which is crucial for traits affected by genotype-environment interactions. Phenotyping of grain crease (*i.e*., crease depth) is another challenge among wheat grain parameters, since the tissues or cells increase regions play influential role in nutrient transportation. Smart Grain itself is not capable to determine crease depth (CD) in wheat grains. These gaps indicate a need for enhanced software algorithms that can adapt to varying grain shapes and environmental conditions, improving the precision of phenotypic data used for QTL and gene discovery (Feng *et al*., 2018).

5. Recent advances in QTL identification of grain size parameters

Recent studies have made considerable progress in identifying QTLs associated with grain size parameters in wheat. Our knowledge of the genetic basis of variables including grain length, width, area, and crease depth has improved as a result of these efforts, which have identified many QTLs that control these characteristics (Wu *et al.*, 2021).

For instance, the identification of QTLs on chromosomes 1B, 2A, and 6A has provided insights into the loci associated with grain length and width under varying environmental conditions. However, translating QTL discoveries into functional candidate genes remains challenging due to the lack of precise phenotypic data and the complex nature of grain size traits.

The complexity of wheat's hexaploid genome further complicates QTL mapping, as multiple homoeologous regions can contribute to a single trait. Recent advances in sequencing technologies and bioinformatics have helped overcome some of these challenges, enabling more detailed mapping and identification of candidate genes. Nevertheless, without precise, high-dimensional phenotyping data, accurately associating QTLs with specific genes remains difficult. Enhanced phenotyping approaches that capture a broad range of grain size-related traits are essential for bridging this gap and facilitating candidate gene discovery. Comprehensive trait measurement is essential for maximizing QTL discovery. Multi-trait analyses enable the identification of pleiotropic QTLs that influence multiple grain size traits, thereby accelerating breeding efforts (Zhang *et al.*, 2016). Studies integrating GL, GW, GAS, and CD into QTL mapping have demonstrated the utility of this approach in improving genetic gain (Fan *et al.*, 2019).

6. Artificial intelligence (AI) and deep learning (DL) in grain size measurement

AI-driven phenotyping platforms use machine learning algorithms to analyze complex trait data with high precision (Ubbens and Stavness, 2017). For instance, convolutional neural networks (CNNs) are used to extract features like CD and CS from high-resolution images, providing scalable solutions for large datasets. These advancements have greatly enhanced the accuracy of phenotypic data and its utility in QTL mapping.

7. Conclusion

High-throughput phenotyping innovations have redefined the potential for analyzing complex traits associated with grain size in wheat, significantly advancing QTL discovery. Technologies like Smart Grain and AI-driven phenotyping models offer unprecedented accuracy and scalability, enabling breeders to characterize multi-dimensional traits like grain length, width, area, and crease depth with precision. While challenges remain, particularly in adapting these models to diverse environments and genotypes, continued advancements in AI and deep learning are likely to overcome these barriers. Enhanced phenotyping not only enables the identification of key QTLs but also supports the development of wheat varieties with improved yield, quality, and resilience, contributing to global food security.

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