Micro-CT-Based Study on Microscopic Phenotypic Characteristics of Maize Kernels (Postprint)

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Abstract

Plant micro-phenotyping primarily refers to phenotypic information at the tissue, cellular, and subcellular levels of plants, and constitutes an important component of plant phenomics research. To address issues such as low efficiency, large errors, and limited indicators in traditional methods for detecting micro-traits of kernels, this study employed Micro-CT scanning technology to conduct precise identification of micro-phenotypes in kernels of 11 corn varieties across 5 types. Based on processing and analysis of CT sequence images, a total of 34 micro-phenotypic indicators were obtained for kernel, embryo, endosperm, cavity, subcutaneous cavity, endosperm cavity, and embryo cavity. Among these, four phenotypic indicators including endosperm cavity surface area, kernel volume, endosperm volume ratio, and endosperm cavity specific surface area showed significant differences among different corn types (P-value<0.05). The endosperm cavity surface area and kernel volume of normal corn were significantly larger than those of other corn types; high-oil corn exhibited the largest endosperm cavity specific surface area; sweet corn showed the smallest endosperm cavity specific surface area; and popcorn displayed the largest endosperm volume ratio. Furthermore, using the 34 corn kernel phenotypic indicators for difference analysis and cluster analysis, the 11 different corn varieties could be classified into four categories, with the first category dominated by normal corn, the second by popcorn, the third by sweet corn, and the fourth by high-oil corn. The results demonstrate that Micro-CT scanning technology can not only achieve precise identification of micro-phenotypes in corn kernels, but also provide technical support for corn kernel classification and variety detection.

Full Text

Study on the Micro-Phenotype of Different Types of Maize Kernels Based on Micro-CT

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Abstract: Plant micro-phenotype primarily refers to phenotypic information at the tissue, cellular, and subcellular levels, representing a crucial component of plant phenomics research. Traditional methods for detecting kernel microscopic traits suffer from low efficiency, large errors, and limited trait coverage. To address these limitations, this study employed Micro-CT scanning technology to conduct precise micro-phenotypic identification of 11 maize kernel varieties spanning 5 common types. Based on processing and analysis of CT sequence images, a total of 34 microscopic phenotypic traits were acquired for seven tissues: whole kernel, embryo, endosperm, cavity, subcutaneous cavity, endosperm cavity, and embryo cavity. Among these traits, four showed significant differences across maize types ($P\text{-value}<0.05$): endosperm cavity surface area, kernel volume, endosperm volume ratio, and endosperm cavity specific surface area. Specifically, the endosperm cavity surface area and kernel volume of common maize were significantly larger than those of other types. High-oil maize exhibited the largest endosperm cavity specific surface area, while sweet maize showed the smallest. Popcorn displayed the highest endosperm volume ratio. Furthermore, differential and cluster analyses based on the 34 phenotypic traits successfully classified the 11 varieties into four distinct groups: common maize (Group I), popcorn (Group II), sweet maize (Group III), and high-oil maize (Group IV). These results demonstrate that Micro-CT scanning technology not only enables precise identification of maize kernel micro-phenotypes but also provides technical support for kernel classification and variety detection.

Keywords: plant phenomics; micro-phenotype; Micro-CT; maize kernel; phenotype identification

1 Introduction

Since the beginning of the 21st century, the rapid development of new-generation information technologies and the advent of the big data era and Breeding 4.0 have transformed agricultural science from traditional theoretical and experimental research into a data-intensive knowledge discovery paradigm, propelling crop science into the omics research era centered on big data. Agricultural basic research in this omics era urgently requires advanced information technologies and automated, high-throughput phenotyping platforms to overcome the limitations of conventional crop phenotyping and analysis techniques. This shift enables in-depth exploration of the intrinsic relationships among phenotype, genotype, and environment from an omics perspective, thereby better serving plant functional genomics and crop molecular breeding research.

Plant phenotyping has been widely applied in crop breeding, molecular biology, and microbiology. Tardieu et al. defined plant phenotype as all identifiable features and traits reflecting plant structure and composition or growth and development processes resulting from genotype-environment interactions. Zhao Chunjiang provided a more systematic and comprehensive definition: plant phenotype encompasses physical, physiological, and biochemical traits that reflect the structure and function of plant cells, tissues, organs, individual plants, and populations. Plant phenotyping spans a vast range, from nucleotide sequences to plant populations, all falling within its research scope.

Micro-phenotype constitutes an essential component of plant phenomics, referring to features and traits at the tissue, cellular, and subcellular levels that reflect internal structure and biochemistry. Micro-phenotype plays a crucial role in precise gene identification and functional prediction. However, compared with phenotyping at the organ, plant, and population levels, micro-phenotype research remains relatively limited and underdeveloped. Achieving comprehensive and rapid acquisition of tissue- and cell-level phenotypic information represents a key research objective in plant phenomics. Addressing the challenges in traditional micro-phenotype research and developing modern micro-phenotyping technologies are critical for meeting current demands, particularly for precise micro-phenotype identification.

Novel imaging technologies, exemplified by X-ray scanning, have significantly advanced plant phenomics and plant developmental biology. Micro-CT (X-ray micro-computed tomography) is a three-dimensional tomographic imaging technique that reconstructs cross-sectional or three-dimensional images of plant tissues based on differential X-ray absorption and transmission rates among different tissues. Originally applied in medical research, Micro-CT has gained increasing attention in agriculture due to its non-invasive, non-destructive, and high-resolution characteristics. In maize research, Micro-CT enables micro-level phenotyping and functional studies, such as extracting three-dimensional information of root vessels to investigate drought resistance relationships or obtaining stem vascular bundle information to study lodging resistance. Combining CT images with automated processing software enables high-throughput acquisition and analysis of maize micro-phenotype data, which can then be integrated with genomic data for association analysis to identify candidate genes related to micro-phenotypes, thereby achieving multi-omics integration.

The maize kernel, as a critical "sink" determining maize yield, contains various physiologically active substances including starch, protein, fat, water-soluble polysaccharides, vitamins, minerals, and essential amino acids. Based on botanical characteristics and biological traits, maize kernels can be classified into multiple types. According to special uses and values, they can be categorized into common maize and specialty maize, with the latter including sweet maize, waxy maize, high-oil maize, popcorn, and other types. The kernel primarily consists of embryo, endosperm, and pericarp. Recent studies have also identified the presence of cavities within maize kernels, which are further classified based on location into embryo cavity, endosperm cavity, and subcutaneous cavity.

Traditional maize kernel phenotyping methods fall into two main categories. The first involves using seed analyzers to obtain conventional RGB images of kernels, enabling analysis of external morphological traits such as length, width, thickness, and density, as well as color and texture features. While RGB imaging offers the advantage of capturing color and texture information, its critical limitation is the inability to provide internal morphological characteristics for studying kernel internal structure. The second method involves preparing paraffin sections of kernels for observation under optical microscopy and image capture, followed by superimposing multiple sectional images to obtain whole-kernel phenotypic features. Although the "sectioning + microscopy" approach can reveal internal structures, the sectioning process is complex and prone to manual and mechanical errors, causing issues such as positional shifts in sequential images that compromise three-dimensional reconstruction and visualization quality. Moreover, sectioning severely damages kernel integrity, preventing subsequent studies.

X-ray tomography-based methods for plant tissue research overcome the complexities of traditional sectioning, enabling non-destructive, three-dimensional tissue imaging and quantitative analysis of three-dimensional phenotypes. Rousseau et al. first employed synchrotron X-ray in-line phase tomography to study the three-dimensional anatomy of maize kernels, validating that X-ray tomography can rapidly acquire spatial structures and segment different internal tissues by adjusting image thresholds and using active contour algorithms. Guelpa et al. used CT scanning to estimate the true density of floury and flint endosperm and whole-kernel density, achieving maize kernel hardness classification and quality grading by combining cavity ratios and porosity. Shao et al. discovered that kernel density and porosity characteristics obtained through Micro-CT were closely related to seed plumpness grading. However, few studies have investigated the micro-phenotypic characteristics of different maize kernel types using Micro-CT.

This study selected 11 varieties across 5 common maize types as research subjects to investigate precise micro-phenotyping methods for maize kernels based on Micro-CT. The objective was to explore trait differences among different kernel types and provide technical support for kernel classification and quality detection.

2 Materials and Methods

2.1 Experimental Materials

Eleven maize kernel varieties across five common types were selected as experimental materials: common maize (B73, Dan 599, Jing 92, Jing 724, MC01), popcorn (830 and 105), waxy maize (Jing Huangnuo 269 and Jing Kenuo 2016), sweet maize (Jing Ketian 608), and high-oil maize (RY732). Three uniformly developed kernels from each variety were selected for subsequent CT scanning.

2.2 Kernel CT Scanning

An X-ray micro-CT system (SkyScan 1172, Bruker, USA) was used for maize kernel scanning. The scanning parameters were: voltage 40 kV, current 250 A, pixel spacing 13.55 m, sample-to-source distance 259.850 mm, and camera-to-source distance 345.591 mm. The system was set to 2K mode (2000$×$1332 px) and performed continuous 180° scanning at 0.4° intervals [FIGURE:1, Step B].

2.3 CT Image Reconstruction

CT scan raw images were reconstructed using CT-Scan NRecon software (Bruker, USA, version 1.6.9.4) to obtain a series of 8-bit BMP format cross-sectional virtual images with a resolution of 2000$×$2000 px [FIGURE:1, Step C].

2.4 Image Segmentation and Phenotypic Trait Calculation

ScanIP image processing software was used for three-dimensional segmentation and phenotypic trait calculation of CT images. The specific procedures were as follows:

(1) Embryo Structure Segmentation. Embryo segmentation was primarily based on grayscale value differences between embryo and endosperm regions in the images, using the Region Growing method. Since grayscale values of embryo and endosperm overlapped, endosperm regions affected embryo segmentation in 3D full-sequence slices. Using single 2D slice images with selective skip-growing operations effectively obtained embryo contours. In this study, 20 images were selected for region growing, followed by close operations to achieve complete embryo segmentation [FIGURE:1, Step D].

(2) Cavity Structure Segmentation. Cavities are internal structures within kernels and can be classified based on location into embryo cavity, endosperm cavity, and subcutaneous cavity. Cavities differ from other structures in both obvious grayscale value differences and structural independence. Using the Region Growing method on the current active mode, the complete embryo cavity structure was obtained. The same procedure was applied for endosperm cavity and subcutaneous cavity segmentation. After obtaining complete structures of embryo cavity, endosperm cavity, and subcutaneous cavity, their modes were summed to obtain the complete total cavity structure.

(3) Endosperm Structure Segmentation. With cavity and embryo structures obtained, the endosperm region (including pericarp) was derived by subtracting cavity and embryo regions from the complete kernel mode, followed by erode operations to remove the pericarp.

(4) Three-Dimensional Phenotypic Trait Calculation. Through steps (1)-(3), three-dimensional segmentation of maize kernel internal structures was achieved, enabling fine segmentation of six different tissues: embryo, endosperm, cavity, subcutaneous cavity, endosperm cavity, and embryo cavity [FIGURE:1, Step E]. Further calculations on each component yielded relevant phenotypic traits including volume (mm^3), surface area (mm^2), mean greyscale (original), volume ratio, and specific surface area [FIGURE:1, Step F].

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3 Results and Analysis

3.1 Three-Dimensional Visualization and Phenotyping of Maize Kernel Tissue Structures

Based on CT sequence images, CT-Analyzer (CTAN) and CT-Volume (CTVOL) were used to render 3D images for qualitative analysis of internal structures, identifying characteristics of different structures and differences among varieties. As shown in [Figure 2: see original paper], three-view images (x-y plane [cross-section], x-z plane [coronal], and z-y plane [rotated sagittal]) of different kernel types and varieties are presented. Combined with ScanIP software, fine three-dimensional segmentation of maize kernel internal structures was achieved, including six different tissues: embryo, endosperm, cavity, subcutaneous cavity, endosperm cavity, and embryo cavity. Further calculations on each component yielded 34 micro-phenotypic traits of maize kernels, as listed in .

3.2 Correlation Analysis of Kernel Phenotypic Traits

To explore relationships among phenotypic traits across different maize kernel types, pairwise correlation analysis was performed on 34 micro-phenotypic traits. Results revealed extremely significant positive correlations ($r>0.94$) between kernel and endosperm in surface area and volume traits [Figure 3: see original paper], indicating that endosperm constitutes the main component of kernels—consistent with the fact that endosperm accounts for over 80% of maize kernel volume. Additionally, correlation coefficients among volumes and volume ratios of total cavity, subcutaneous cavity, and endosperm cavity were all greater than

0.8 (extremely significant positive correlation), suggesting that cavity size is primarily influenced by subcutaneous and endosperm cavities.

Correlation analysis of mean greyscale (original) among different kernel tissues showed that correlation coefficients among embryo, endosperm, and whole kernel were all greater than 0.90 with similar greyscale values, indicating similar tissue densities among kernels, endosperm, and embryo, and that kernel density is significantly correlated with internal endosperm and embryo tissues.

3.3 Differential Analysis of Kernel Phenotypic Traits

To explore relationships of the same phenotypic trait across different kernel types, one-way ANOVA and LSD tests were performed using R.

3.3.1 Differential Analysis of Whole Kernel Traits Ranking kernel volumes of 11 varieties revealed that common maize varieties (Jing 92, B73, Jing 724) had significantly larger volumes than sweet maize (Jing Ketian 608), high-oil maize (RY732), and popcorn (105, 830). Jing 92 exhibited the largest volume (251.67 mm^3), significantly different from specialty maize ($P<0.05$) . However, common maize kernel volume could not be distinguished from waxy maize, likely due to their similar external morphological phenotypes.

Specific surface area (kernel surface area-to-volume ratio) is an important morphological indicator. The ranking of specific surface area among 11 varieties was: Jing Ketian 608 > RY732 > 105 > Jing Kenuo 2016 > Jing Huangnuo 269 > 830 > Dan 599 > B73 > MC01 > Jing 724 > Jing 92, indicating that specialty maize generally has larger specific surface area than common maize. For example, sweet maize Jing Ketian 608 had the maximum specific surface area (2.59), significantly different from other kernels, while the smallest specialty maize specific surface area (830, 1.49) was still larger than the largest common maize specific surface area (Dan 599, 1.43) . These results demonstrate that kernel volume and specific surface area can differentiate specialty maize from common maize.

3.3.2 Differential Analysis of Internal Structures Among Kernel Types To further compare internal structural differences among kernel types, one-way ANOVA was performed on phenotypic traits of embryo, endosperm, cavity, subcutaneous cavity, endosperm cavity, and embryo cavity. As shown in , among the top 5 varieties for endosperm volume, 4 were common maize (Jing 92, B73, Jing 724, MC01), with Jing 92 having the maximum endosperm volume (210.67 mm^3). Specialty maize (popcorn 105, high-oil maize RY732, sweet maize Jing Ketian 608) had significantly smaller endosperm volumes, with Jing Ketian 608 having the minimum (83.7 mm^3).

Ranking endosperm cavity surface areas showed that 5 common maize varieties ranked in the top 7 positions (Dan 599, Jing 724, B73, MC01, Jing 92), with endosperm cavity surface areas ranging from 88.73 to 132.40 mm^2, significantly higher than other types (4.79-54.24 mm^2) . Conversely, ranking by embryo volume ratio revealed that all common maize varieties (except Dan 599) had embryo volume ratios of 9-15, significantly smaller than specialty maize such as sweet maize Jing Ketian 608 (25.19) and high-oil maize RY732 (24.11) . These analyses indicate that endosperm volume, endosperm cavity surface area, and embryo volume ratio can differentiate common maize from specialty maize.

Endosperm volume ratio analysis showed that popcorn varieties 105 and 830 ranked top two among all types, both >87 , indicating that popcorn kernels have larger endosperm volume proportions than other types. Additionally, endosperm cavity specific surface area could differentiate high-oil maize RY732 from sweet maize Jing Ketian 608. RY732 had the maximum endosperm cavity specific surface area (97.44), while Jing Ketian 608 had the minimum (11.06), significantly different from other types . Endosperm cavity volume ratio also distinguished these two types, with Jing Ketian 608 having the maximum (7.33) and RY732 the minimum (0.05) .

3.3.3 Differential Analysis of Mean Greyscale Mean greyscale values were also analyzed. As shown in , cavity structures (embryo cavity, endosperm cavity, subcutaneous cavity) had mean greyscale values below 50, while non-cavity structures (embryo, endosperm, kernel) had values above 80, with significant differences between cavity and non-cavity tissues. This indicates that mean greyscale can differentiate cavity from non-cavity structures and serves as an important phenotypic indicator for tissue classification.

3.5 Phenotypic Trait Cluster Analysis

K-Medoids cluster analysis was performed on 34 micro-phenotypic traits using the pam() function in R’ s cluster package. Results in [Figure 4: see original paper] show that 11 kernel varieties can be divided into four groups: Group I (Jing 724, MC01, Jing Huangnuo 269, B73, Dan 599, Jing 92; blue), Group II (Jing Kenuo 2016, 105, 830; green), Group III (Jing Ketian 608; yellow), and Group IV (RY732; red).

One-way ANOVA on these four groups revealed that 8 traits showed significant differences (P-value<0.05): endosperm volume, kernel volume, endosperm cavity surface area, embryo cavity surface area, kernel specific surface area, endosperm cavity specific surface area, embryo volume ratio, and endosperm volume ratio . LSD tests based on these 8 traits and 4 groups showed that embryo cavity surface area did not differ significantly among groups and was thus unsuitable for variety differentiation. Therefore, the remaining 7 traits were used for classification.

Group characteristics based on mean trait values were: Group I had maximum endosperm cavity surface area, endosperm volume, and kernel volume, with kernel volume significantly different from other groups; Group II had maximum endosperm volume ratio (though not significantly different from Groups I and

IV at P<0.05) and minimum embryo volume ratio (though not significantly different from Group I); Group III had minimum endosperm volume, endosperm cavity specific surface area, and endosperm volume ratio, but maximum kernel specific surface area and embryo volume ratio; Group IV had minimum endosperm cavity surface area and kernel volume, but maximum endosperm cavity specific surface area.

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4 Conclusion and Discussion

This study investigated micro-phenotypic characteristics of 11 maize varieties across 5 types (common, waxy, popcorn, high-oil, and sweet maize) using Micro-CT. The main conclusions are:

(1) Micro-CT scanning enabled three-dimensional segmentation of 7 tissue structures (kernel, embryo, endosperm, cavity, subcutaneous cavity, endosperm cavity, embryo cavity) and precise quantification of 34 phenotypic traits.

(2) Among the 34 traits, 4 showed significant differences among maize types (P-value<0.05): endosperm cavity surface area, kernel volume, endosperm volume ratio, and endosperm cavity specific surface area. Common maize had significantly larger endosperm cavity surface area and kernel volume than other types. Popcorn had the highest endosperm volume ratio. High-oil maize had the largest endosperm cavity specific surface area, while sweet maize had the smallest.

(3) Differential and cluster analyses based on 34 traits successfully classified 11 varieties into four groups: Group I (Jing 724, MC01, Jing Huangnuo 269, B73, Dan 599, Jing 92), Group II (105, 830, Jing Kenuo 2016), Group III (Jing Ketian 608), and Group IV (RY732). This classification aligns well with actual maize types, demonstrating that Micro-CT scanning can support variety classification.

The current CT image segmentation workflow involves substantial manual interaction, making it time-consuming and inefficient for high-throughput micro-phenotyping of maize kernels. Future research will focus on improving the efficiency and accuracy of micro-phenotype acquisition for large-scale kernel studies.

Waxy maize differs from common maize primarily in biochemical composition rather than external morphology, which explains why Jing Huangnuo 269 was grouped with common maize in cluster analysis. Among specialty maize, sweet maize Jing Ketian 608 had the smallest endosperm cavity specific surface area, significantly different from other types . Conversely, high-oil maize RY732 had the largest endosperm cavity specific surface area with significant differences, consistent with cluster analysis results where RY732 and Jing Ketian 608 were separately classified [Figure 4: see original paper]. Popcorn varieties 105 and

830 had higher endosperm volume ratios than other types , and LSD tests on the 8 significantly different traits showed that Group II (mainly popcorn) had higher endosperm volume ratios than other groups , indicating that endosperm volume ratio is an important trait for differentiating popcorn. Similarly, kernel traits such as kernel volume and endosperm cavity surface area showed large inter-type differences and could differentiate common from specialty maize. For example, ranking endosperm cavity surface area and kernel volume showed that the top 50% were all common maize varieties [TABLE:4 and TABLE:3], consistent with cluster analysis where common maize formed a distinct Group I [Figure 4: see original paper]. Both differential and cluster analyses identified endosperm cavity surface area, kernel volume, endosperm volume ratio, and endosperm cavity specific surface area as significantly different traits among maize types, providing important references for classification.

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Note: Figure translations are in progress. See original paper for figures.