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Fast analysis of Maize Kernel Plumpness Characteristics through Micro-CT Technology

Meng Shao^{1, 2, 3, §}, Ying Zhang^{1, 2, 3, §}, Jianjun Du^{1, 2, 3}, Xiaodi Pan^{1, 2, 3}, Liming Ma^{1, 2, 3}, Jinglu Wang^{1, 2, 3}, Dennis BÖhmer⁴ and Xinyu Guo^{1, 2, 3} (✉)

¹ Beijing Key Lab of Digital Plant, Beijing, China

² Beijing Research Center for Information Technology in Agriculture, Beijing, China

³ Beijing Academy of Agriculture and Forestry Sciences, Beijing, China

{shaom, zhangying, dujj, panxd, malm, wangjl, guoxy}@nrcita.org.cn

⁴ University of Bonn, Bonn, Germany

s6deboeh@uni-bonn.de

Abstract. Maize plumpness believed to reflect yield and quality of maize products. Convenient and accurate methods may help identification of maize quality for produces and germplasm resources for breeding. In this study, the 3D reconstruction of maize kernel based on micro-CT technology was introduced to detect anatomical difference between diverse classes of maize kernel. Void spaces measurements constructed for whole maize (*Zea mays* L.) kernel gives a more accurate volume measurement for density calculations by means of a package of commercial softwares. Moreover, the ratio of cavity and porosity of the entire kernel were calculated based on the 3D CT images. Kernel density, cavities, porosity, and other phenotypic characteristics were closely related to seed plumpness classification. Compared with previous methods, our method significantly improves the calculation accuracy of kernel volume, cavity and porosity, and which is expected to be useful for efficient maize kernel plumpness classification.

Keywords: micro-ct; image segmentation; kernel plumpness; porosity; cavity.

1 Introduction

Maize occupies an important position in food economy in the world. It is also a major food resource and an important industrial material in China. The quality of the maize kernel reflects the grain yield and the variety adaptability. Maize kernel plumpness is an important phenotypic trait believed to reflect yield and quality of maize products [1-2]. Qualitative and quantitative analysis of maize kernel plumpness is most important prerequisite for studying genetic factor, physiological process and improvement methods of maize [3-6]. In previous study, indicators such as density, test-weight, specific gravity, and hardness or others [7-10] were as equated factors for kernel plumpness analysis. However, it was time consuming and hard to measure the kernel density and other indicators in traditional methods and simple, rapid and reliable methods to analysis kernel plumpness are greatly needed. In maize kernel, different compartments exist and enclose each other and the developments of these compartments have much influence in maize kernel quality [11]. During the development of the maize kernel, the whole size of kernel and various components within the kernel change dramatically. The morphological structure and final size of endosperm represent the accumulation of nutrient storage, determine the kernel plumpness. Vitreous and floury endosperm are two types of endosperm both present in maize kernel [12]. There is void space in kernel because of starch granules less compacted in floury endosperm [13]. Furthermore, air cavities form in maize kernel due to the endosperm decomposes to form a space when the kernel dehydration [14-15].

Measurements that void spaces will give a much accurate volume for density calculations ^[4]. However, traditional density test methods, such as floating test, pay no attention to the cavities in the kernel. Meanwhile, it is demonstrated that the porosity and cavity volume ratio of the kernel showed a strong correlation with maize quality and reflected the compactness of starch granules in endosperm ^[16-17].

X-ray micro-CT is now considered has the advantage to perform non-destructive, non-invasive and three-dimensional visualization and quantification of the internal structures of biology material, which the resolution can get to 1 μm ^[18, 19]. In recent years, this technology was used to visualize the above-ground structures of plants ^[20-25] and mainly focused on air void spaces ^[26-27] and global 3D structure inside the plant tissues ^[28]. A lot of researches in the food field have been published ^[29]. Chawanji ^[30] used the CT technology to research the micro-composition of different milk powder. Donis-González ^[31] visualized the inner features of fresh chestnut based on micro-CT. The micro-CT also applied to the internal quality evaluation and porous of food produces ^[32-33]. However, these studies have not referred to the quantitative research of the internal characterization. Quantitative techniques are urgent need to accurately characterize micro traits of kernel, in order to establish the relationship between micro-phenotyping and the seed quality.

[§] These authors, Meng Shao and Ying Zhang who contributed equally to this work.

2 Material and Method

2.1 Material

Two groups maize kernels were randomly selected from each inbred line, X178 (CK) and W99 (not released as a commercial inbred line), different in maize kernel quality. These kernels were harvested from plants which planted at experimental field in Beijing academy of agriculture and forestry science on May 5, 2016.

2.2 Non-destructive micro-CT of maize seed

Kernels were selected random and then whole kernels were scanned by using X-ray micro-CT system (SkyScan 1172, Bruker Corporation) respectively. The scan parameter was 40 KV/250 μ A. The object-to-source distance was 182.700 mm and the camera-to-source distance was 215.601mm for every scan. The sample was rotated for 180° on the sample stage with images every 0.4°. After scanning, the raw CT data in 2K (2000×1332 pixels) were reconstructed into 8-bit sequence images using mathematical algorithm (such as Feldkamp). These CT slice pictures resulted in a 3D stack with the grey value calibrated from 0 to 255. The result file was imported CT-volume ('CTVol') program (Bruker Corporation) to visualize entire spatial architecture of maize kernel. The CTVol was used to reconstruct 3D models of maize kernel internal structure that could be visualized in pseudo colors.

2.3 Image processing

The image processing flow for segmentation of embryo, endosperm and cavity of kernel used the Simpleware software version 7.0 (Simpleware LTD, UK<http://www.simpleware.com>) was given in Fig. 1. And the small pores of the floury endosperm were segmented by CTand software (Brucker Company). Due to different parts have different grey values, different image operations could be used to segment different parts of kernel. The cavity could be segmented by simple "flood fill" and "region grow" processing. The grey value of embryo was so close to the vitreous endosperm that it was too difficult to segment these two parts by "region grow" on global 3D image. However, used "region grow" method with the following parameters number of iteration=1, multiplier=2, the initial neighborhood radius=1 were suitable for the segmentation of the embryo on the 2D selection slices of X-ray images (Fig. 1). After region growing of these selection slices, the "close" step (Fig. 1) was operated on the all consecutive slices to the segmentation of embryo (Fig. 1). The endosperm segmentation was subtracted the embryo and cavity from the entire seed.

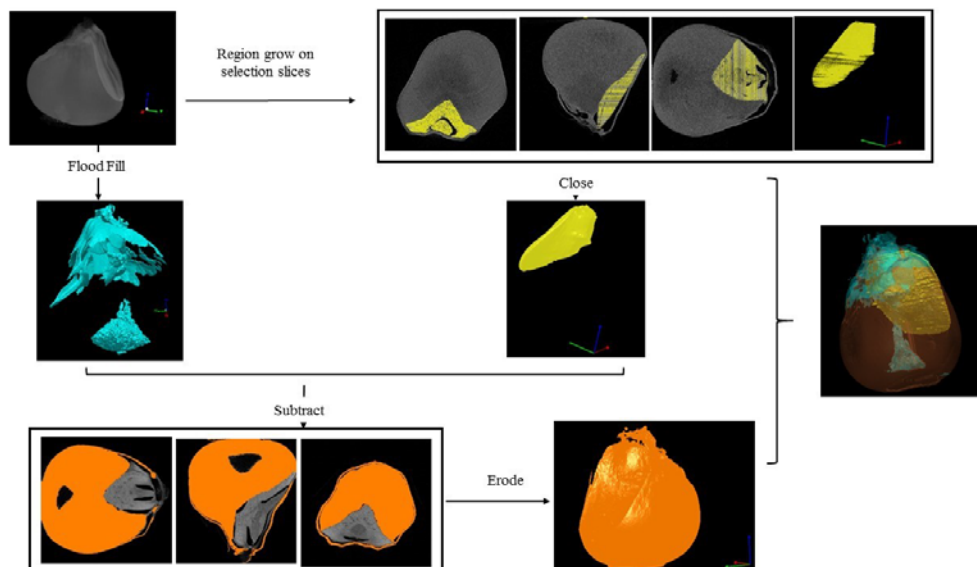


Fig.1. Image processing flow for the segmentation of embryo, endosperm, and cavity of maize kernels using Simpleware software based on micro-CT 3D image

2.4 Data processing

The respective measurements (% cavity, % porosity) were compared averages with respect to plumpness classes by the One-way analysis of variance (ANOVA).

3 Result and Discussion

3.1 2D and 3D images of maize kernel

In 2D analysis, shows Fig. 2, the both two group seeds in the 2D CT slices which taken longitudinal images to visualize the morphology and different internal compartments of maize seed. A multiphase composition of the maize kernel was observed. The kernel was made up the pericarp, embryo, endosperm and the air cavity. In 2D image slices, different parts could be differentiated through the different absorption value of X-ray. Vitreous and floury were two types of endosperm in kernel. The vitreous endosperm (brighter, Fig. 2.) was harder with higher density outside of the kernel compared with the floury endosperm (darker, Fig. 2.). Due to the starch being less compacted in floury endosperm, many air cavities were present. From the contrast of bright and dark, surrounding air and internal void space was black, embryo was bright. Large cavities in the floury endosperm of both the two group seeds were observed in all repeated tests, but much more prominent in the inbred line X178 seeds. Compared of CT images, differences of plumpness for the two maize seeds were apparent (Fig. 2.).

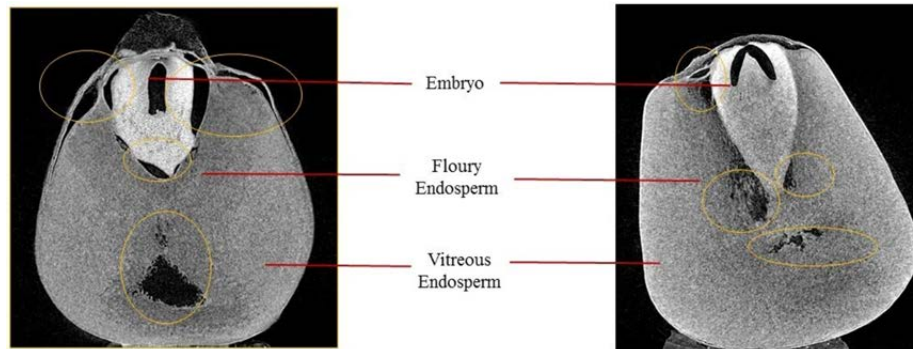


Fig. 2. 2D CT images of the different group maize kernel X178 (left) and W99 (right). Cavities was marked with yellow circles

For the both group seeds, the image resolution was 1220×1220 pixels and the maximum resolution up to 6.91 μ m. Before image reconstruction, fine tuning was done to reduce noise and correct detector defects by NRecon software, including post-alignment, beam-hardening correction, ring-artifacts reduction and smoothing. The visual 3D models were reconstructed through CTVox software and could be reviewed in various planes by Dataview software packages (Fig. 3.).

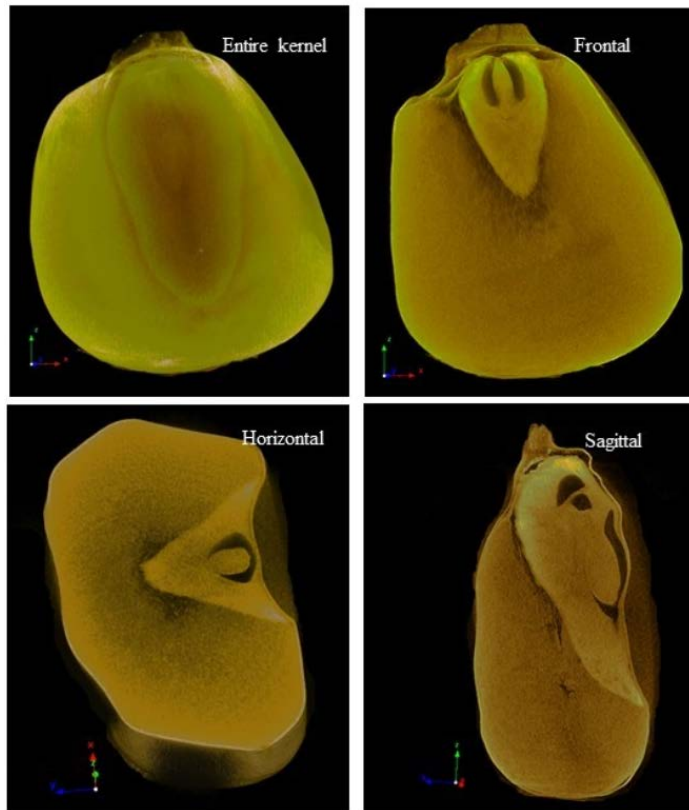


Fig. 3. 3D reconstruction and visualization of maize kernel in different planes with pseudo colors using NRecon and CTvox software

3.2 Image segmentation

In maize kernel, the void space and the surrounding air region had the same low grey value. Thus the ROI (region of interest) was used to restricted to internal the maize kernel pericarp (include). The cavity structures with high contrast were segmented by simple “Flood fill” and “region grow”. Skipping slices of the CT images, the selection slices on 2D image of embryo were segmented by region grow operation. The embryo segmentation was obtained after closing all the slices of embryo. For endosperm segmentation, the pericarp was separated from the mode by “erode” operate after using global mode subtracted the cavity and embryo parts. CTan software were used to extract small pores in floury endosperm accurately. First, removed part of the seed point which was almost empty and had much damage in skin and reduced random noise to find the surface of the kernel accurately. The pores were distinguished from the entire kernel by thresholding. The ROI was restricted to the surface of maize kernel using shrink-wrap to measure the volume of the whole maize kernel. The embryo, the pericarp and the cavity between embryo and pericarp were segmented into different ROI in the sample based on morphological differences and density variation in a sample. The floury endosperm was also segmented from endosperm due to the different density of floury and vitreous endosperm. The porosity (%) of the floury endosperm was obtained by a 3D analysis.

3.3 Quantitative analysis

Once different ROIs have been segmented, a segmented volume and defined measurements could be performed. The volumes of different compartments (modes) were given by the software. The kernel true volume was calculated accurately by measurement the eliminate pores and cavity space (Eq.):

$$\text{Kernel true volume} = \text{Total VOI volume} - \text{pores volume} - \text{cavity volume}$$

All ten scanned kernels (each group selected 5 kernels randomly and measured the mass of each kernel by laboratory scale) were extracted volume of cavities, pores and the kernel true volume from the CT image data by image processing above. Fig.4. showed the 3D CT images of the two maize kernels with small pores visualized in red. The cavities (%) of all ten kernels were quantified respectively and which results were depicted in Table 1. The cavities (%) of the W99 inbred line were significantly lesser than that of the X178 inbred line (Table 1). The porosity (%) of X178 kernels was $0.94\pm 0.09\%$, however, the W99 inbred line was significantly lower, which porosity (%) was only $0.49\pm 0.07\%$. Furthermore, based on the 3D models, the kernel true volume, cavity traits of kernels from different inbred lines were calculated, which results were as shown as Table 1. These phenotypic traits, including kernel true volume, porosity (%) were closely related to the plumpness of maize kernel, which might be the important indicators of seed quality identification.

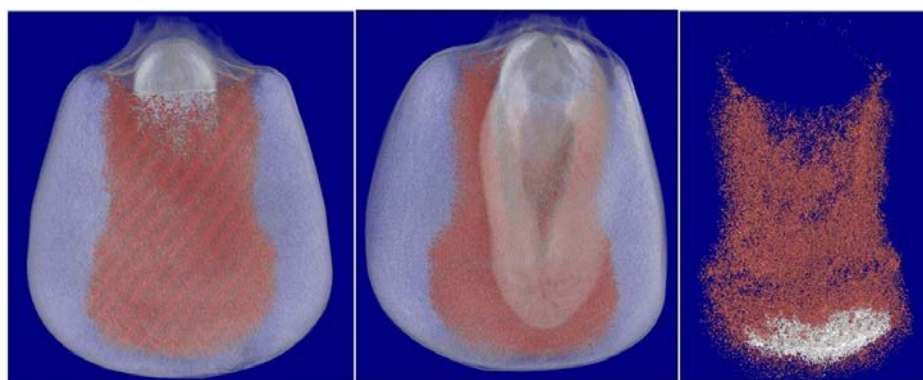


Fig. 4. 3D analysis of the porosity of maize kernel (small pores in red)

Table 1. Quantitative index of maize kernel for different inbred lines

Trait	W99	X178	P values
Porosity (%)	0.49 ± 0.071	0.94 ± 0.09	<0.01
Cavities (%)	1.53 ± 0.26	3.83 ± 0.64	<0.01
Kernel true volume (mm^3)	269.5 ± 3.40	218.3 ± 5.20	<0.01

4 Conclusions

The biggest advantage synchrotron radiation based CT technology is high quality of scanned images, which is more effectively to disclose inner features of plant tissue and the sample can be as small as 5~10 mm [34-36]. However, the synchrotron radiation based X-ray scanning technology is not suitable for mass of agriculture products studies in standard lab. New development in modern desktop micro-CT system based on cone-beam geometry accessible to standard lab and provide high quality image [28, 35]. The cone beam geometry has the advantage in taking into account the thickness of the object and the rays going through the front and back of the object will not be projected on the same row of the detector. In this study, we introduce desktop micro-CT technology to realize the 3D reconstruction of different parts morphology internal maize kernel in standard lab environments. Different parts of the maize kernels were differentiated through grey values of 3D micro-CT images [19]. It demonstrates that the plump maize kernel forms less cracks in the maize kernel than the less plump one by visualized the longitudinal of the 2D CT images. X-ray micro-CT is also applicable to determinate true volumes and densities of the sample. Guelpa et al [17] calculated the maize kernel density by polymers used as calibration using CT. In order to make sure the linear function is suitable for the kernel density collation,

the polymer discs were taken along with the kernels stacked on top of each other in every scan. However, this method was too limited and time-consuming to obtain the density of mass of maize kernel products. Over the past decade, morphological quantitative analysis software has improved greatly and commercially image processing software was the demand of agriculture [35]. Currently, a number of commercial softwares are available for extracting information from CT data for qualitative and quantitative analysis. The quantitative techniques can obtain analysis information such as volume, porosity, thickness from the sample. In this study, the kernel true volume and different compartments of the maize kernel can obtain from Simpleware and CTan commercial software with analyzing data in batch processing for the same class of sample. Eliminating void spaces gives a more accurate volume measurement for density calculations. Thus, we consider that it is a simple, rapid and reliable method to calculate a more accurate density of maize kernel. Additionally, desktop micro-CT is demonstrated the feasibility to quantify the microscopic phenotypic traits of kernel such as cavities and porosity. And these important phenotypic parameters can be used as an important indicator of seed plumpness analysis.

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