Kernel position effects of grain morphological characteristics by X-ray micro-computed tomography (μCT)

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Abstract: Grain size and shape are important factors for yield and quality. The difference in grain phenotypic characteristics in the same maize hybrid is related to its position in the ear. This study aimed to clarify the distribution characteristics of grain morphological characteristics in the ear and to provide guidance for research of grain phenotype and kernel position effects. Three maize hybrids were used in the experiment, namely, Denghai 618 (DH618), KX3564, and Xianyu 335 (XY335), and the kernel number per row were 40, 40, and 36, respectively. The X-ray μCT was applied to obtain five kernel morphological indicators, including grain length, width, thickness, volume, surface area. Grain sphericity, length-width ratio, specific surface area, and volume coefficient were further calculated. The results showed that there were three types of maize ear morphological indicators trends: grain length, width, volume, and surface area were parabolic; thickness and sphericity were inverted parabolic; length-to-width ratio and specific surface area were irregular. The volume coefficient of grain at different parts of the ear, namely the relation coefficient between grain volume and grain length, width, and thickness, was determined. The average value of the middle grains morphological indicators of the ear was taken to select kernels representing stable characteristics of the variety. Within the range of 5% deviation from the morphological mean value of the middle grains of the ear, the grains in the middle part accounted for 26.39% of the total ear, about 10 grains extending from the 14th grain at the base of the ear to the top. Within the range of 10% deviation, the middle accounted for 47.22%, about 18 grains extending from the 12th grain at the base of the ear to the top. This study found that grain morphological indicators were greatly different at different positions of the maize ear, and showed different change rules as extend from the base to the top of the ear. Therefore, there were different grain volume coefficients at different positions of maize ear. And the representative sampling range on maize ear was determined based on the comprehensive analysis of different morphological indexes variation of grain.
Keywords: grain morphology, X-ray μCT, kernel position effects, maize (*Zea Mays* L.)
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1 Introduction

Grain size and shape are important factors in improving yield potential and quality[1]. And grain size and uniformity are also important targets of commercial quality. The grains show great differences in shape and internal composition due to the different positions in the ear[2-4]. After fertilization of the female ear florets in maize, the grain development was best in the middle and lower parts of the ear, followed by the base and then the top, which was the worst[5-7]. Maize quality is best in the middle of the ear, and middle seeds have stronger genetic potential and can best represent genetic characteristics of the variety[8,9]. It has been reported that compared with the kernels in the middle of the panicle, the number of endosperm cells in the upper part of the panicle was small, and the activities of enzymes such as sucrose degrading enzyme and starch synthase were weak[6]. Zhao et al.[10] found that there was no significant difference in the dry weight, fresh weight and volume of seeds in the middle and lower parts of the ear, while there was a significant difference between the upper and middle and lower parts of the ear. Previous studies on the kernel position effect mainly focused on the mechanism of kernel development and abortion, and the differences of grain quality and germination characteristics in different parts of the ear were also involved[11,12]. At present, crop phenotypic studies have also begun to pay attention to the phenotypic characteristics of grains. However, there are few studies on phenotypic information such as grain size

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and shape at different positions of the ear.

The kernel size and shape, such as the grain length, width and thickness, grain sphericity and length-width ratio, etc., are important agronomic traits of maize[13]. Grain morphological characteristics reflect the process of grain filling to a certain extent. Grain weight is commonly used to describe maize characteristics in production, while some studies have shown that grain weight is closely related to grain size and volume[13,14]. Large-grain wheat contributes to increasing germination vigor and grain yield[15]. For example, breeders are more inclined to choose large seeds than small seeds[16]. Reynolds et al.[17] believed that seed size was a more feasible target for future yield improvement through genetic modification. Vyn et al.[18] showed that small and rectangular maize grains were more resistant to breakage after analyzing the breakage rates of five hybrid varieties at different drying temperatures after harvest. In our previous work, it was found that the crushing resistance of maize was related to the size, sphericity and texture of maize grain[19]. It could be seen that grain morphological characteristics were highly valued by breeding and quality researchers and are widely used in production. However, few people have studied the rules of grain morphological indexes at different positions of the ear due to the lack of accurate measurement tools.

Traditionally, the morphological characteristics of grain length, width and thickness are usually measured by hand. The grain volume is typically measured by the displacement method with a large error[20]. Meanwhile, the hundred kernel weights were used to reflect kernel size, but it could not measure real kernel volume. Grain characteristic indexes, such as specific surface area, sphericity and grain density, were not sufficiently accurate due to grain shape irregularity. With the research and application of new technology, the method of grain morphology measurement has been greatly improved. Neuman et al.[21] used digital image technology to detect the spatial shape and Fourier transform the circumference of wheat kernels to calculate the general outline of the kernels. The machine vision ImageJ plug-in developed with Java determines the vertical length and width of grains from digital images[22]. The characteristics of grain size, shape and color were studied by computer image analysis and recognition[23]. Zhang et al.[24] designed a computer image processing system with a digital camera to determine the shape parameters, such as the projected area, long axis and short axis, of wheat and achieved automatic measurement of the appearance shape parameters of wheat. Liu et al.[25] compared and observed the microscopic morphological characteristics of the seed coat, seed umbilicus and umbilicus coronae of wild soybean by scanning electron microscopy. Walker et al.[26] used 2D digital images to determine the size, weight, volume and density of a single barley grain.

X-ray μCT is a new method of data acquisition, because it can quickly and accurately reconstruct the surface morphology of 3D objects, such as individual kernel volume surface area and size, and has the characteristics of high precision, high speed and 3D scanning. Because of nondestructive, 3D visualization and quantitative analysis, X-ray μCT enables 3D microstructure investigation of samples in a near-native state and at unprecedented resolution, increasingly widely used[27-29]. The absorption of X-rays is also different depending on the internal composition of the grain, according to the differences in pixel values, the kernel density and volume of maize can be obtained by X-ray μCT[30]. Thang et al.[31] also observed changes in grain morphology during the development of wheat by X-ray μCT. Previously, it was difficult to investigate grain structure and characteristics such as volume, density, sphericity and internal structure parameters. X-ray μCT enables us to observe the structure of grains in a three-dimensional, non-destructive manner[32-34]. The internal and external parameters obtained by X-ray μCT scanning technology also provide a new method for the research of yield traits (such as single-grain weight), quality traits (such as bulk density, hardness and nutrient content), grain crushing resistance, and grain dehydration.

There was a great difference in the shape of maize kernels at different positions. This research applied X-ray μCT to obtain eight kernel morphological indicators of three maize hybrids: grain length, width, thickness, volume, surface area, grain sphericity, grain length-width ratio, grain specific surface area, and grain volume coefficient, etc. Aims to clarify the rules of grain morphological characteristics in different parts of the ear, and provide guidance for research of grain phenotype and kernel position effects.

2 Materials and methods

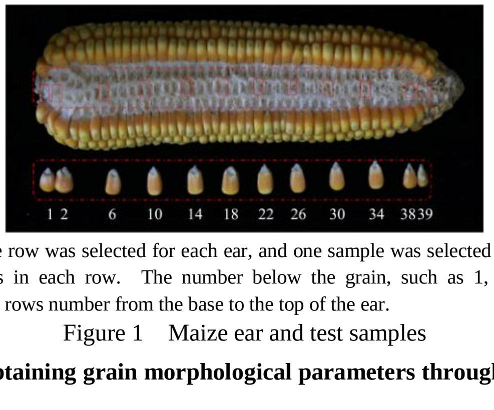
2.1 Test varieties and test samples

Three maize hybrids were used in this study, namely, Denghai 618 (DH618), KX3564 and Xianyu 335 (XY335), the per-row kernel numbers of the ear of DH618, KX3564 and XY335 maize were 40, 40 and 36, respectively. Variety information is shown in Table 1. The experiment was arranged in Qitai County, Xinjiang Region. All plants in the experiments were subjected to the same planting density, water and fertilizer management.

Table 1 Information of the three maize hybrids DH618, KX3564 and XY335

Varieties	Female parent	Male parent	Registration NO.	Panicle type	Grain type
DH618	521	DH392	Guoshenyu20176113	barrel panicle	half-dent type
KX3564	KW4M029	KW7M14	Ningshenyu2010004	barrel panicle	dent type
XY335	PH6WC	PH4CV	Guoshenyu2004017	barrel panicle	half-dent type

Considering that a large number of 2D images were obtained after X-ray μCT scanning, test samples were selected intermittently from the base to the top of each ear, and one grain was selected every 1-3 grains. The morphological characteristics of grains near the base and top of the panicle varied greatly, and the sampling amount was increased at both ends of the panicle. On average, 12-15 grains were selected from each ear (Figure 1).



Note: One row was selected for each ear, and one sample was selected for every 1-3 grains in each row. The number below the grain, such as 1, 2, 6, etc represents rows number from the base to the top of the ear.

Figure 1 Maize ear and test samples

2.2 Obtaining grain morphological parameters through X-ray μCT

X-ray μCT scanning equipment and parameters are shown in

Table 2. Detailed CT scanning methods can be referred to as Reference [19]. During the scanning process, the sample is rotated on the translation table while illuminated with X-rays (Figure 2). The CT data in the 4K scan module were converted into a series of CT slice images with an 8-bit tagged image file format using Skyscan NRecon software (Bruker Corporation).

Table 2 X-ray μCT scanning equipment and parameters

X-ray μCT equipment	Source-to-object distance /mm	Source-to-image distance /mm	X-ray source	Scan module	Taken frequency /(°)	Exposure time /ms	Pixel size /μm
Skyscan 1172	259.850	345.591	tungsten	4K	/	/	/
Charge-coupled device (CCD) camera	/	/	/	/	0.2	1475	7

Note: 40 kV/250 mA tungsten.

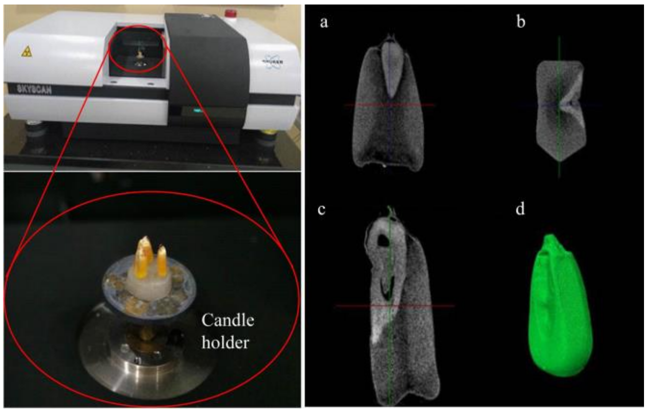


Figure 2 Skyscan 1172 X-ray computed tomography system and three-dimensional structure reconstruction of grains

More than 900 2D images (*x-y*, *x-z*, and *y-z*) of different sections of the grains were obtained by scanning the grains for 15 min. The 2D images of different angles of the grain sample obtained from X-ray μCT Scan were used to reconstruct three-dimensional by CT-Analyser (CTAn) and CT-volume (CTVol) software (Bruker Corporation, Germany). So the entire grain spatial architecture was analyzed and visualized (Figure 2). Generally, the more scanned images that were obtained, the more accurate the 3D reconstruction and surface recovery of the ear but the higher the computational cost and the lower the efficiency[35]. The CTAn and CTVol software were used to reconstruct CT raw images that were subsequently visualized in color. The length, width and thickness of grains were measured with the ruler tool of CTVol software (Figure 3). The main indexes of maize grain morphology obtained are shown in Table 3.

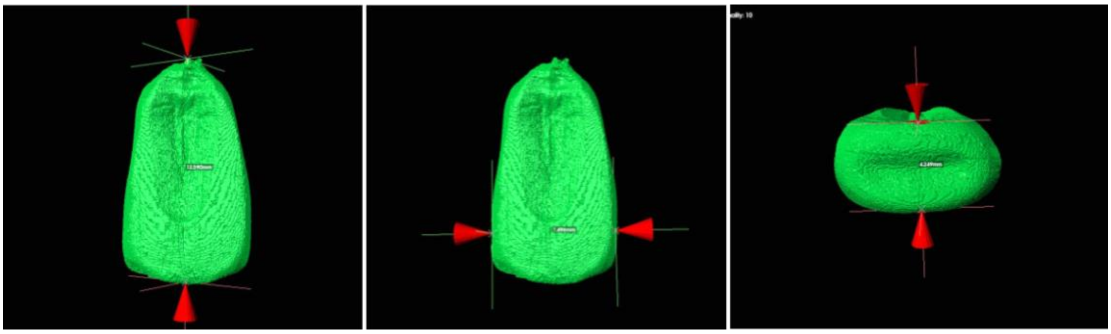


Figure 3 Grain length, grain width, and grain thickness

Table 3 Morphological parameters obtained by X-ray μCT

Parameters	Features	Illustration
Length (*L*)/mm	Top-to-bottom distance of the 3D image	Grain length
Width (*W*)/mm	Two-side distance of the 3D image	Grain width
Thickness (*T*)/mm	Distance between the embryo and endosperm of the 3D image	Grain thickness
Grain volume (Vg)/mm^3	Whole volume of the grain 3D image	External volume of grain; represents the grain storage capacity
Grain surface area (SAg)/mm^2	Whole surface area of the grain 3D image	External surface area of a grain

2.3 Calculation of grain shape

$$\text{Grain sphericity} = 4\pi\left(\frac{3Vg}{4\pi}\right)^{\frac{2}{3}} / SAg \quad (1)$$

$$\text{Grain length} - \text{width ratio} = L / W \quad (2)$$

$$\text{Grain specific surface area (mm}^2/\text{mm}^3) = SAg / Vg \quad (3)$$

where, grain sphericity, grain length-width ratio and grain specific surface area are grain morphological parameters to characterize the grain shape.

2.4 Statistical analysis

Statistical analysis was performed using Microsoft Excel 2010 and IBM SPSS Statistics 19.0 (SPSS Inc., Chicago, IL, USA). The data were smoothed by filtering by Origin 8.5 software. The data interpolation method was used to supplement unmeasured data[36].

3 Results

3.1 Morphological characteristics of grains at different positions in the ear

3.1.1 Grain length

The grain length at the base of the ear was smaller and then gradually increased to the maximum, remained stable in the middle of the ear, and decreased rapidly to the minimum near the top of the ear (Figure 4). Among the three varieties, DH618 had the largest and smallest grain length. Thus, the variation range for grain length was the largest for DH618. The position where the maximum grain length was reached was the 10th grain from the base of the ear for XY335, 14th grain for DH618, and 22nd grain for KX3564. The grain positions with a deviation within 5% from that of the maximum grain length for DH618 were 7-26, accounting for 50% of the grain number per ear. KX3564 was 6-29, accounting for 60.00%, XY335 was 7-27, accounting for 58.33%. And the grain range and the proportion in the ear deviating from the maximum grain length by 10% of the three varieties are respectively 4-32 (72.50%), 3-33 (77.50%) and 4-31 (77.78%).

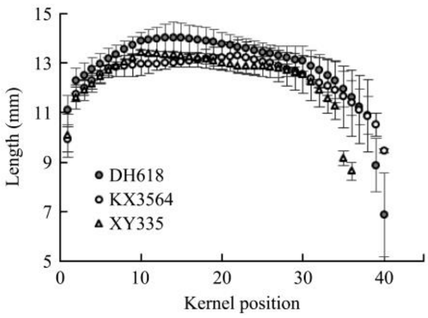


Figure 4 Grain length at different positions of the ear

3.1.2 Grain width

The grain width at the base of the ear increased to the maximum and then gradually decreased slightly; near the top, the grain width dropped rapidly to the minimum, and all three varieties exhibited the same trend. In terms of the maximum grain width, that of the KX3564 variety was the largest, the position of which was reached the earliest at the 6th grain from the base of the ear. XY335 exhibited the smallest grain width, and the maximum width of the grain reached the latest at the 10th grain from the bottom of the ear (Figure 5). The positions with a 5% deviation from the maximum grain width for DH618, KX3564 and XY335 were 3-23, 2-24 and 4-20, accounting for 52.50%, 57.50%, and 47.22% of the ear respectively. The range of grain positions with 10% deviation and the corresponding proportion in the ear of the three varieties are respectively was 2-30 (72.50%), 1-28 (70.00%) and 2-31 (83.33%), respectively.

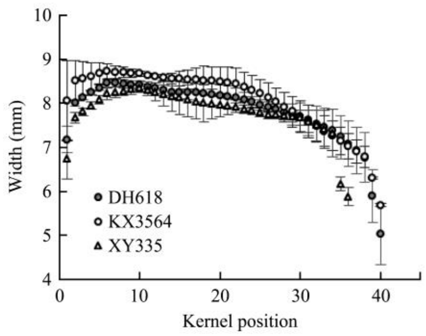


Figure 5 Grain width at different positions of the ear

3.1.3 Grain thickness

The kernel thickness change in maize ear was an inverted parabola. The kernel thickness at the base was the largest, that at the middle was the smallest, and at the top, it increased. The trend was observed for all three varieties. In terms of minimum kernel thickness, the thicker one observed was DH618, at the 26th grain from the base of the ear, and the thinner one was XY335, at the 22nd grain from the base of the ear (Figure 6). The range of positions exhibiting a 5% deviation in minimum grain thickness for DH618 was 13-32, accounting for 50% of the grain number per ear. KX3564 was 11-27, accounting for 42.50% of the ear. XY335 was 18-26, accounting for 25.00%. The range in grain positions and the corresponding proportion in the ear exhibiting a 10% deviation from the minimum grain thickness were 10-34 (62.50%), 9-30 (55.00%) and 15-28 (38.89%), respectively.

3.1.4 Grain volume

Except for several grains at the base and top, the change in grain volume at different positions basically linearly decreased from the top to the base of the ear. The smallest grain volume occurred at the top of the ear. There were also differences in the positions of the largest grain volume among the different varieties. DH618 and KX3564 exhibited maximum grain volumes at the 6th grain from the base of ears, which was slightly earlier than that of XY335 (Figure 7).

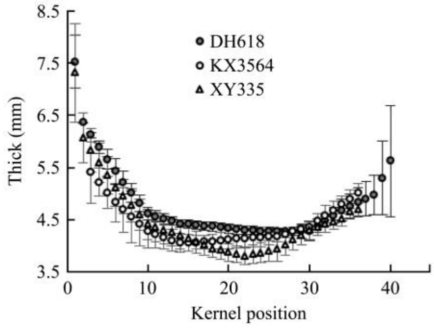


Figure 6 Grain thickness at different positions of the ear

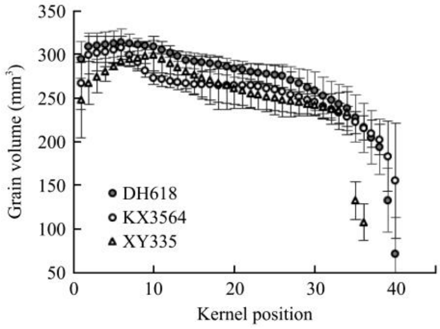


Figure 7 Grain volume at different positions of the ear

3.1.5 Grain surface area

In terms of grain surface area, that of grains at the base was bigger and the top was smaller, moreover, there was great variation in grain surface area among the different varieties. At the base, the surface area of grains of KX3564 changed rapidly, reached the maximum surface area value at the 6th grain from the base, and then decreased gradually. Grains of XY335 reached the maximum surface area at the 10th grain from the base, and those of DH618 reached the maximum surface were at the 18th grain (Figure 8). The kernel positions of the three varieties with a deviation of 5% from the maximum surface were 3-22, 2-12 and 4-18 for DH618, KX3564 and XY335, accounting for 50.00%, 27.50%, 41.67% of the ear respectively, and the kernel ranges and the corresponding proportion in the ear with a surface area deviation of 10% from the maximum value were 2-28 (67.50%), 1-26 (65.00%) and 2-23 (61.11%), respectively.

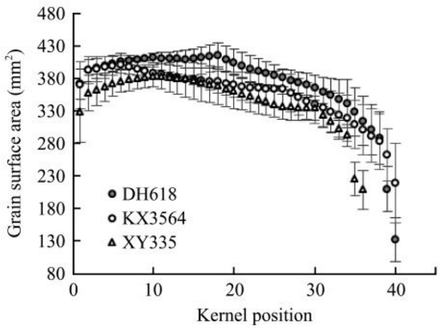


Figure 8 Grain surface area at different positions of the ear

3.1.6 Grain sphericity

The variation in grain sphericity was similar to an inverted parabola. The grain sphericity was relatively large at the base, gradually reached the minimum value in the middle, and then gradually increased to the maximum value at the top of the ear. There were consistent changes among the three varieties. For the minimum value of grain sphericity, that of XY335 grains was the largest among the three varieties and occurred at the 20th grain from the base of the ear. The smallest minimum sphericity was of DH618 grains, which was reached at the 18th grain from the base of the ear (Figure 9). The kernel positions and the corresponding proportion in the ear with 95% of the minimum grain sphericity for DH618, KX3564 and XY335 were 11-28 (45.00%), 3-27 (62.50%) and 6-29 (66.67%), respectively, and those with 90% of the minimum value, DH618 were 3-27, accounting for 87.50% of the grain number per ear. KX3564 was 1-34 85.00%. XY335 was 1-36, including the whole ear.

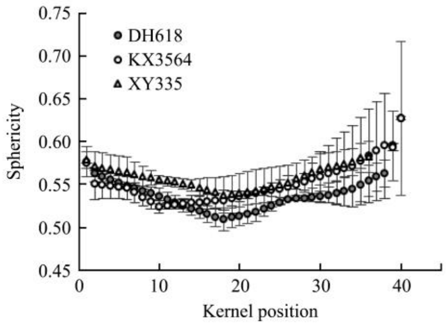


Figure 9 Grain sphericity at different positions of the ear

3.1.7 Grain length-width ratio

The ratio of length to width of grains in the ear varied greatly among varieties. That of DH618 and XY335 grains gradually increased from the base and then slowly decreased, while KX3564 increased until it reached the top of the ear. The grain position corresponding to the maximum grain length-width ratio varied greatly among the different varieties; those of DH618 and XY335 were largest in the middle of the ear, while that of KX3564 was largest at the base (Figure 10).

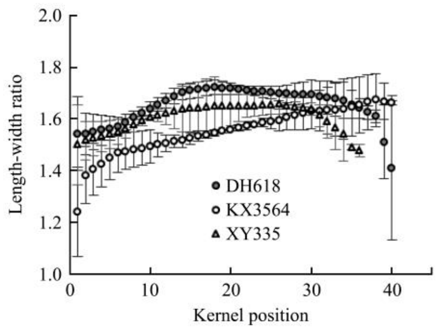


Figure 10 Grain length-width ratio at different positions of the ear

3.1.8 Grain specific surface area

The specific surface area of the grains of the three varieties increased from the base, and that of grains in the middle part of the ear was relatively stable. However, the range in grain specific surface area at the top of the ear was quite drastic. The largest specific surface area was observed for DH618 (Figure 11).

In summary, three varieties with the same ear type (barrel type) and different grain types (dent type and half-dent type) were used. There were three types of kernel morphological indicators trends with different positions on maize ears. The length, width, volume and surface area of maize kernels exhibited a parabolic trend, with the characteristics of the smaller basal and apical grains and larger middle grains, and the minimum values usually appeared at the top of the ear. The thickness and sphericity exhibited an inverted parabolic trend. The length-width ratio and specific surface area exhibited irregular trends that were difficult to describe. It could be speculated that the methods of calculation contributed to their variation and complexity. The maximum and minimum values of grain characteristics of different varieties were different, and the corresponding grain positions were different for the maximum values. The change rule of grain morphology index is different in the ear, but the performance of maize varieties is the same.

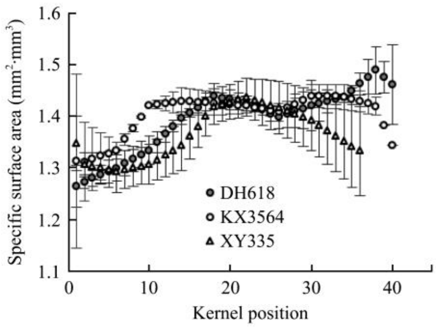


Figure 11 Grain specific surface area at different positions of the ear

3.2 Relationship among grain morphology indexes

Among the three factors of grain type: length, width and thickness, grain length was highly significant positively correlated with grain width and negatively correlated with grain thickness. Grain width and grain thickness were negatively correlated. In terms of grain volume, grain width and grain length had the greatest influence and showed a significant positive correlation, while grain thickness had less influence on grain volume at different ear positions. Although there is a certain difference in the correlation coefficient between the indexes, the correlation is consistent among the varieties.

Table 4 Correlation analysis of grain morphology indicators

Varieties	Indicators	*L*/mm	*W*/mm	*T*/mm	V_g/mm^3
DH618	*L*	1	0.905**	–0.451**	0.862**
	W		1	–0.189*	0.939**
	T			1	0.027
	Vg				1
KX3564	*L*	1	0.691**	–0.710**	0.588**
	W		1	–0.433**	0.885**
	T			1	–0.171
	Vg				1
XY335	*L*	1	0.848**	–0.442**	0.750**
	W		1	–0.228*	0.800**
	T			1	0.138
	Vg				1

Note: ** and * represent significant correlations at the levels of 0.01 and 0.05, respectively.

3.3 Grain volume coefficient of maize

Because of the irregular shape of the grains, the grain volume cannot be simply multiplied by the length, width and thickness of the grains. The analogy to the leaf shape coefficient (k=0.75), the grain volume coefficient (VCg) can be calculated according to the accurate grain morphological data obtained by X-ray μCT.

$$VCg = Vg/(L \cdot W \cdot T) \quad (4)$$

The grain volume coefficient in the middle of maize ear was relatively stable and higher than that at the base and top of the ear. And three varieties exhibited the same trend. In the middle of the ear, the volume coefficient of XY335 is slightly higher than that of the other two varieties, while DH618 and KX3564 have little difference in the grain volume coefficient in the middle of the ear.

The variation range of grain volume coefficient of DH618, KX3564 and XY335 from the base of the ear to the top was 0.43-0.61, 049-0.59 and 0.45-0.66, respectively (Figure 12).

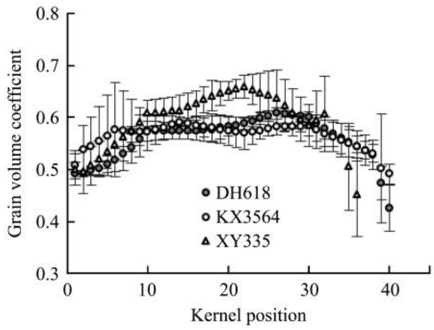


Figure 12 Grain volume coefficient at different positions of the ear

3.4 Determination of representative sampling range in the ear

The variation of grain shape parameters in the middle of the ear was small, and the grains selected from the middle ear extending to both ends of the ear were more uniform. The method based on the mean value of grain morphology indicators in the middle of the ear is easy to be realized in sampling. Comprehensive analysis of the eight morphological indicators of three varieties, it was considered to have little or no variation that when the deviation was within 5% or 10%, which as a criterion to evaluate the range of representative kernels in an ear of the variety.

Based on the above criteria, within 5% of the deviation from the grain morphology indicators in the middle of the ear, it showed that the middle parts accounted for 26.39% of the number of grains per ear, at least 36.11% grains of the base and at least 37.50% grains of the top should be omitted, that is about 10 grains extend from the 14th grain at the base of the ear to the top. Moreover, if the sampling standard were relaxed to within a 10% deviation from the middle grain of the ear, the middle part accounts for 47.22% of the whole ear. Now, it could be proposed to remove at least 27.78% grains of the base and 25% of the top when sampling, about 18 grains extend from the 12th grain at the base of the ear to the top (Table 5).

Table 5 Positions range of representative grain in the ear

Parameters		Varieties					
		DH618		KX3564		XY335	
		5%	10%	5%	10%	5%	10%
L/mm		6-30	3-33	6-29	3-33	6-30	3-32
W/mm		2-28	2-34	1-26	1-30	2-30	2-34
T/mm		12-32	10-34	10-29	9-31	14-28	11-30
V_P/mm^3		14-27	8-30	10-27	8-31	14-23	11-31
S_P/mm^2		2-25	1-30	11-28	1-31	2-23	2-30
L/W		10-36	3-38	9-34	4-40	8-32	1-35
S		9-32	2-38	2-31	1-37	6-29	1-36
$S_P\ V_P^{-1}$/mm^2 mm^{-3}		12-40	5-40	7-39	1-40	15-34	1-36
VC		9-34	7-38	4-35	2-38	14-27	9-34
Common	Kernel position	14-25	10-30	11-26	9-30	14-23	11-30
	Kernel numbers	12	21	16	22	10	20
	Proportion/%	30.00	52.50	40.00	55.00	27.78	55.56
The base/%		32.50	22.50	25.00	20.00	36.11	27.78
The top/%		37.50	25.00	35.00	25.00	36.11	16.67
Selected/%		37.50 (the top)	25.00 (the top)			36.11 (the base)	27.78 (the base)
Middle proportion/%		26.39 (5%)				47.22 (10%)	

Note: The middle ear of DH618 and KX3564 were the average values of the grain morphology indicators of 20th-21th grains in ear and XY335 was the average values of the 18th-19th grains in ear. Maximum base ratio and maximum top ratio were selected from three varieties, so as to determine stable middle area satisfying three maize hybrids.

4 Discussion

The differences in grain morphological characteristics were a comprehensive performance of grain gene expression, grain filling, the activity of synthetase involved in chemical substances and physical factors at different positions of the ear. Kernel position effects on morphological characteristics are also prevalent in wheat[37], rice[38,39] and other cereal crops, especially in large-panicle varieties[2,40]. In this study, dent type and half-dent type maize varieties were selected, and grain morphological characteristics were measured by X-ray μCT. It was found that there were significant differences in grain morphological characteristics among varieties, and the basic change in grain characteristics was consistent among varieties. The length, width, volume and surface area of grains in the middle part of ears of the three varieties were larger than those of grains in the basal and top parts, while the grain thickness and sphericity were significantly smaller in the middle than in the basal and top parts. The reasons for the morphological differences between the grains in the ear may be roughly divided into two sides: one view is that there are natural developmental differences between the grain positions of the female panicle[41]. The top grain storage capacity is smaller than that of the middle and basal grains. Another view is that the lack of source is the cause of abortion at the top of the panicle. Factors such as photosynthesis or pollination control can indirectly affect the development of maize grains at different positions[42,43]. From a physical point of view, the grain shape on the ear of maize is determined by the spatial pressure of three dimensions, namely, the number of ear rows, the cob circumference and the tightness of bracts. The grains in the middle of the ear compete with each other, so the grains are flat with a small thickness, and grains are more spherical at both ends of the ear.

In this study, correlation results of grain morphological traits found that the grain volume of different maize varieties was mainly affected by grain length and width. Although there were some differences in correlation coefficients among the indexes, the correlation effects were consistent varieties. It is consistent with the results of Zheng's study on the relationship between kernel growth and size in maize[44]. This work can provide a theoretical basis for maize breeders to breed maize varieties to meet specific needs. Besides, the grain volume coefficient is different due to different positions of the ear. In the middle part of the ear, the grains are rectangular, and the grains at the base and top are almost spherical. The conversion from grain length, width and thickness to grain volume can be realized at different positions of the ear, according to grain volume coefficient (Table 6).

Table 6 Grain volume coefficient at different positions of ear

Deviation from the middle of the ear	Grain position	DH618	KX3564	XY335
5%	Middle part	0.58	0.58	0.64
	Base and top	0.55	0.56	0.57
10%	Middle part	0.59	0.58	0.63
	Base and top	0.53	0.55	0.55

There is no ear sampling range representing the characteristics of the variety of maize. Xu et al.[6] classified the grains at the top of an ear (approximately 1/3 of the total length of the ear) as weak grains and those at the middle and bottom of the ear (approximately 2/3 of the total length of the ear) as strong grains. Ou-lee et al.[7] showed that the region of fertilized kernels on each ear measuring 80% to 90% of the distance from base to tip of the ear. The basal kernels were from the region 10% to 20% of this distance. Lu et al.[45] modified the method of Ou-lee slightly by dividing the ear into three segments, the base, top and middle, accounting for approximately 20%, 20% and 60% of the ear, respectively. In this study, the per-row kernel numbers of an ear of DH618, KX3564 and XY335 maize were 40, 40 and 36, respectively. Within the range of 5% deviation from the morphological mean value of the middle grains of the ear, the grains in the middle part accounted for 26.39% of the total ear, the base accounted for 36.11%, and the top accounted for 37.50%; Within the range of 10% deviation, the middle accounted for 47.22%, the base accounted for 27.78% and the top for 25%. The differences in grain morphological characteristics at different positions were the external manifestations of the differences in grain gene expression, grain filling and activity of synthetase involved in chemical substances at different positions on the ear. Therefore, it is reasonable to segment the ear according to grain morphological characteristics.

5 Conclusions

X-ray μCT was adopted to obtain grain morphological indicators. There were some differences in the effect of grain position on morphological characteristics among the different varieties, but the overall rule was the same. The morphological indexes of different positions of the ear can be divided into three types: grain length, width, volume and surface area that were parabolic, thickness and sphericity were inverted parabolic and length-to-width ratio and specific surface area were irregular. Meanwhile, the relationship between grain volume and grain length and grain width, namely grain volume coefficient, was determined. Comprehensive analysis of the variation trend of different kernel morphologies in maize ears, within the range of 5% deviation from the morphological mean value of the middle grains of the ear, the grains in the middle part accounted for 26.39% of the total ear, about 10 grains extend from the 14th grain at the base of the ear to the top. Within the range of 10% deviation, the middle accounted for 47.22%, about 18 grains extend from the 12th grain at the base of the ear to the top.

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