Phase states of moisture content in different maize kernel types

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Abstract: Accurate determination of the moisture content in maize kernels conduces to screen maize germplasm materials with efficient dehydration. Low-field nuclear magnetic resonance (LF-NMR) single-kernel non-destructive testing technology was used to determine the moisture content at different phase states in the kernels for selected types of maize. The NMR T_2 relaxation inversion spectrum was monitored in maize kernels to determine the variation in the moisture content in different phase states with time. The total water and free water peaked at the filling stage of the maize kernels and then declined to a minimum at physiological maturity. The semi-bound water generally increased to a long-lasting peak in the dough stage and then declined. The bound water increased from kernel formation to maturity and then remained stable. The contents of total water, free water, semi-bound water, and bound water had significant differences among kernel types but not among varieties of the same type. The contents of semi-bound water and free water were linearly correlated with the dehydration rates of the kernels. The results of this study can provide a means for creating new germplasm materials.

Keywords: maize (*Zea mays* L.), LF-NMR, moisture phase state, kernel type, dehydration rate
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1 Introduction

Grain moisture content is a key factor affecting the mechanical harvest quality, safe storage, and economic benefits of maize. Maize is the most important cereal crop and economic crop in China. Research and development on maize agronomy focus on realizing the harvest of good quality kernels and mechanization of maize production[1,2]. The kernel moisture content of maize at harvest is determined by the kernel moisture content at physiological maturity and the kernel dehydration rate after physiological maturity[3], which determine the patterns of moisture content in maize kernels. Cultivating maize varieties with low moisture content and strong dehydration ability may enhance grain harvest, and shorten the drying time of kernels after harvest, which prevents mildew, and improve maize quality[4].

The moisture content of kernels during harvest is an important factor affecting maize yield, especially in mechanical harvesting. Already research investigated agronomic traits related to kernels' moisture content and dehydration rate at harvest, such as leaf moisture content at harvest, number of functional leaves at flowering, ear diameter, bract traits, kernel type and size, pericarp permeability, ear height[5-9]. However, these studies are limited by evaluations based on a single index of moisture content, ignoring differences in phase state and properties of the moisture content of kernels, where moisture is a nutrient carrier for plants[10]. The moisture contents of different phase types (bound water, semi-bound water, and free water) have different effects on kernel activity[11,12]. Bound water, as a component of cell structure, is an indispensable component for maintaining life activities. Bound water combines with proteins, polysaccharides, and other substances in cells, which cannot dissolve other substances, and does not participate in material metabolism. Free water has high solvents flowing freely within cells, between cells and organisms, which can dissolve a variety of substances and compounds, and participate in material metabolism. The free water increases or decreases with changes in external humidity and temperature[13,14]. After harvesting, the bound water in maize kernels cannot be utilized by microorganisms, whereas free water can be utilized. Therefore, maize kernels with higher free water are more likely to rot or breed mold[15]. The moisture content of different phase states during harvesting affects the storage quality of maize.

In addition, there are differences in the contents of various nutrients in different maize kernel types at different developmental stages[16]. Differences in the hydrophilicity of different nutrients may lead to differences in the contents of different types of water in kernels[8]. As the carrier of carbon assimilates transport in maize kernel, moisture controls the filling and maturity of kernels and is also directly related to the complex physiological and biochemical processes inside the kernel. Different proportions of phase states affect the physical properties of the cell protoplasm and the intensity of metabolism[17].

Low-field nuclear magnetic resonance (LF-NMR) is a non-destructive technology for testing that can be used to investigate the changes in the phase state and distribution of moisture in samples[14]. This technology has been widely used in agriculture and the food industry[18-23] with the advantages of non-invasive, fast, and simple applications. LF-NMR can be used to detect the occurrence and migration patterns of phase states of moisture in kernels during drying and soaking[2,24]. According to the deviations of individual glucose isotopologues, the flux patterns of the central carbohydrate metabolism were determined during kernel development in maize[25]. LF-NMR can rapidly and accurately measure the moisture and oil content in fried starch systems[26] to distinguish the development of endosperm texture in wheat kernels[27]. LF-NMR can also characterize the changes in moisture phase state and content among germplasm materials to provide an important means for improving germplasm, for example, to allow machine harvesting, and for creating new germplasm

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materials[28]. However, to the best of the authors' knowledge, there has been no research on the application of LF-NMR for detecting the phase state of moisture in maize kernels during growth.

In this study, LF-NMR technology was used to dynamically monitor the internal moisture content of living maize kernels. The patterns and differences in the contents of different phases of moisture were examined among different kernel types and different varieties of the same kernel type, and the effects of kernel type on the moisture content and dehydration rate of maize kernels were explored. This study provides theoretical support for breeding new varieties suitable for mechanical harvest and has practical importance for guiding production.

2 Materials and methods

2.1 Test equipment

Testing was performed using NMI20-015V-I nuclear magnetic resonance instrument (Magnet type: permanent magnet, magnetic field strength: (0.50±0.05) T, RF pulse frequency: 12.2 MHz, magnet temperature: 32°C, probe coil diameter: 15 mm), a Mettler-ToledoXS105 DualRange analytical balance (Precision: 0.001 g, measuring range: 120 g), a drying box (GZX-9246 MBE), a digital camera (Canon EOS 90 (D)18135ISUSM), and a Vernier caliper.

2.2 Plant materials

Maize can be divided into nine types according to their morphology, endosperm structure, and glume. The types that were selected for this study are mainly used as food, feed, and industrial materials; and they have similar growth periods. The four types used in this study were as farinaceous type (Liaodan145), dent type (Liaodan1281), flint type (Liaodan502), and semi-dent type (Xianyu335 and Liaodan586). Table 1 lists the characteristics of different maize varieties.

Table 1 Characteristics of different maize varieties

Varieties	Plant height/cm	Ear length/cm	Ear diameter/cm	Rows	Types	HGW/g	Yield/kg·hm^{-2}	Crude protein content/%	Crude fat content/%	Crude starch content/%	Lysine content/%	Growth stage
Liaodan145	306	21	6.8	16-20	Farinaceous	39.6	9682.5	10.91	4.74	73.49	0.32	134
Liaodan1281	312.9	27.5	5.4	18	Dent	38.85	12174	11.20	3.27	73.31	0.29	129
Liaodan502	270.0	24	5.4	16-18	Flint	42	8866.5	11.62	4.43	69.93	0.34	135
Xianyu335	313.0	19.5	5.2	16-18	Semi-dent	34.3	14001.0	9.55	4.08	74.16	0.30	135
Liaodan586	270	25	5.5	18	Semi-dent	40.6	12151.5	10.99	3.37	73.28	0.31	136

2.3 Test design

The test was conducted at the Shenyang Agricultural University experimental base (41°49'N, 123°33'E). The first field trial was sown on May 12, 2019, and harvested on October 10, 2019. A random-block design was adopted, with three repeats for each variety giving a total of 15 plots. Each plot was 10 m long and 6 m wide (10 ridges, ridge spacing 0.6 m), giving a plot area of 60 m^2 and a test area of 900 m^2. The planting density was 60 000 plants/hm^2, the planting row spacing was 60 cm, and the plant spacing was 30 cm. Samples were picked every 3 d starting 10 d after pollination, twenty times in total. Two ears with the same appearance and uniform size were selected in the same plot per variety of maize for each sampling. Two maize kernels with a desired morphology were selected from the middle of each sampled maize ear, giving a total of 12 kernels per variety per sample time for analysis. Each test group had one kernel from each variety, and 12 groups of parallel test samples were prepared at each of the twenty sample times.

The second field trial was sown on May 14, 2020, and harvested on October 15, 2020. The planting and sample collection standards were as described for the first test.

2.4 Data acquisition

There are three stages in the development of maize kernels from double fertilization to physiological maturity, namely filling, dough, and physical maturity stages[29]. The filling stage is 0-16 d after pollination when the moisture content is approximately 80%-90%; the dough stage is 16-40 d after pollination when the moisture content decreases to 40%-80%; and the mature stage is 40-55 d after pollination when the kernel moisture content decreases to 25%-40%. In this study, the maize kernels of Liaodan145, Liaodan1281, Liaodan502, Xianyu335, and Liaodan586 were selected as experimental samples for information collected from the 10th day after maize pollination onwards, according to the 20 times nodes of the experimental design. Table 2 lists the relationships between all collection time nodes and the maize development stages.

Table 2 Relationships between all collection time nodes and the maize development stages

Reproductive stage	Time node	Measure times
Filling stage	1-3	3
Dough stage	4-13	10
Physiological Maturity stage	14-20	7

After silking, the sampling procedure at each time node was to select the listing marks of maize with basically the same growth, break off the samples, and measure them. The maize-related agronomic traits that were measured included single grain weight (fresh weight and dry weight) and hundred-grain weight. The test protocol was as follows. Taking the fresh weight of a single grain (W_1), then measuring the relaxation spectrum by LF-NMR, and finally measuring the moisture content using the traditional drying method (test samples were pretreated of high temperature desiccation under 105°C dried at 80°C to constant weight). The dry weight (W_2) of the maize kernel was measured, and the moisture content of the test samples was calculated as follows:

$$\text{Moisture content} = \frac{W_1 - W_2}{W_1} \times 100\% \tag{1}$$

2.5 LF-NMR detection program

LF-NMR is a detection technology that has the advantages of being non-invasive and non-destructive[30]. Nuclear magnetic resonance T_2 relaxation spectroscopy is an effective method for the analysis of plant moisture to detect changes in moisture distribution and content in living plants. Differences in NMR transverse relaxation time T_2 of water molecules provide a measure of moisture in different micro-regions of plant tissue and allow the analysis of the phase state of moisture in the plant tissue[15,31].

2.5.1 NMR spectroscopy test

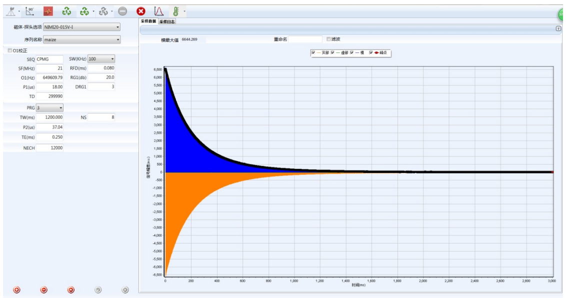
The protocol for the NMR spectroscopy test was as follows. Using a standard oil sample matched with the equipment, complete a free induction decay (FID) hard pulse sequence test to determine the center frequency (SF1+O$_1$) and pulse width of NMR. The parameters in this test were the main value of the RF signal

SF_1=12.2 MHz, a pulse width of RF 90°C P_1=18 μs, and a pulse width of RF 180°C P_2=36 μs. The test sample was placed horizontally into the bottom of the glass tube, and the glass tube was inserted into the center of the LF-NMR. The carr-purcell-meiboom-gill pulse sequence (CPMG) was used to collect the NMR signal and test the transverse relaxation process of the sample. The mathematical expression of the relaxation signal is as follows:

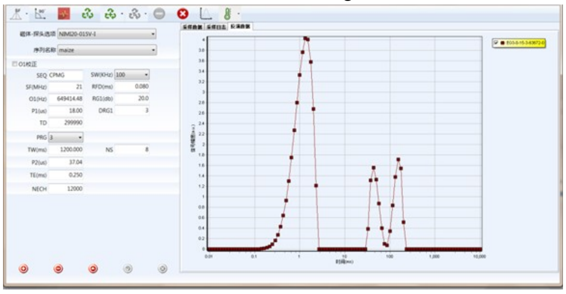
$$M(t)=\sum_i P_i \exp\left(-\frac{t}{T_{2i}}\right) \tag{2}$$

where, $M(t)$ is the signal quantity after the transverse magnetization vector decays to time t; P_i is the signal intensity of the i^{th} component, the total signal is the sum of the signal generated by all components in the test sample; T_{2i} is the transverse relaxation time of the i^{th} component in the sample.

The parameters of the CPMG pulse sequence are set as receiver bandwidth SW (kHz) of 100, sampling points TD of 299 990, RF delay RFD of 0.08 ms, repeated sampling interval TW of 1200 ms, cumulative sampling times NS of 8, echo number NECH of 1200. Each test sample was tested three times.



a. Data collecting



b. Data inversion

Figure 1 Process of data collecting

2.5.2 Analysis of data

The mean value of the NMR data of each sample was calculated, and the mean value was taken as the NMR signal amplitude of the test sample. Microsoft Excel 2013 was used to process the basic data. The quality of all kernels was uniform (0.3 g), and because the volume and weight of maize kernels at different growth stages were different, the data of peak area of relaxation inversion spectrum, moisture content, and dry matter weight could not be compared directly. Laplace inversion was performed to obtain the T_2 inversion spectrum data for a combination of discrete and continuous NMR. According to the different parameter settings in the pre-test, the main parameters were as follows: for multiple groups, the minimum relaxation time was 0.01 ms, the maximum relaxation time was 10 000 ms, and there were 100 relaxation time points and 100 000 iterations.

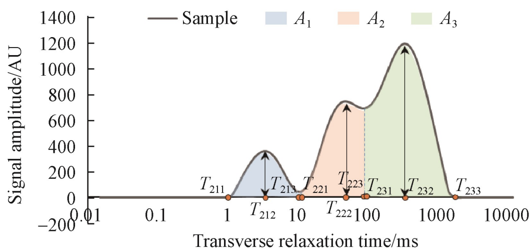
The relaxation characteristics of the test samples including peak start time, peak vertex time, peak end time, peak area, peak proportion, and total area, were for the different time points by inversion fitting. The inversion data were imported into SPSS 25.0, for processing and analysis, including ANOVA, correlation analysis, and Student's T-test.

2.5.3 Analysis of T_2 inversion spectrum results

According to the principle of nuclear magnetic resonance, the T_2 relaxation time is positively correlated with the degree of freedom of protons in the maize kernel. The longer the relaxation time, the higher the degree of freedom of the water molecule. Therefore, the relaxation time indirectly indicates the phase characteristics of moisture[12]. The signal amplitude (peak area) of the inversion spectrum represents the relative content of hydrogen protons in each interval, to distinguish and measure the relative content of moisture in different phase states. The three-phase states of moisture in the maize kernel have distinct multi-component characteristics in the NMR T_2 relaxation spectrum.

Based on the fitting curve of T_2 relaxation data obtained from test samples, we found that the T_2 relaxation time of test samples of each group ranged from 1 to 1000 m with an obvious boundary between 10 ms and 100 ms. The different phase states of moisture were defined according to the characteristics of the relaxation spectra of the maize kernel. The critical boundary between bound water and semi-bound water was taken as 10 ms, and the critical boundary between semi-bound water and free water was taken as 100 ms.

The three peaks in the inversion spectrum of relaxation time in maize kernels represent three different moisture phase states. The start, peak, and end times of the signal are T_{2i1}, T_{2i2}, and T_{2i3} (i=1, 2, 3), respectively, which represent the transverse relaxation time node of the i^{th} component in the sample. The area A_i of each peak and abscissa represents the signal intensity of component i, that is the moisture content of each component. The short transverse relaxation time was set to T_{21} (i=1, T_{21}=T_{213}−T_{211}), and the water molecules in this part were defined as bound water. This fraction of moisture is closely bound to other macromolecules through hydrogen bonds and is adsorbed on organic solid materials. This type of moisture is insoluble in other substances and has low fluidity, which corresponds to the relaxation inversion spectrum signal amplitude A_1. The moisture in the part of the longer transverse relaxation time, set to T_{23} (i=3, T_{23}=T_{233}−T_{231}), was defined as free water. Free water exists in free form and has strong fluidity, corresponding to the relaxation spectrum signal amplitude A_3. The transverse relaxation time between bound water and free water was set as T_{22} (i=2, T_{22}=T_{223}−T_{221}), and the water molecules in this part were defined as semi-bound water. The corresponding relaxation inversion spectrum signal amplitude is A_2. A was used to express the total water content of the maize kernel, A=A_1+A_2+A_3. The proportion of water content in different phases of maize kernel can be obtained by T_2 relaxation data, so the moisture content in each phase state can be obtained by multiplying the measured kernel moisture content by the scale factor, as shown in A_1, A_2, and A_3 in Figure 2.



Note: The start, peak, and end times of the signal are T_{2i1}, T_{2i2}, and T_{2i3} (i=1, 2, 3), respectively.

Figure 2 Inversion spectrum of transverse relaxation time of one maize kernel

As an example, Figure 2 shows the data from a test sample (Xianyu335) that was collected at the time node on September 10, 2019, which is the transverse relaxation time inversion spectrum after inversion.

3 Results and analysis

3.1 Analysis of moisture changes in four grain types during growth period

3.1.1 Analysis of changes in phase state of moisture content

The changes in total moisture content and the relative moisture content of the different phase states, from the filling stage to the harvest stage are shown in Figure 3 for kernels of four different maize types.

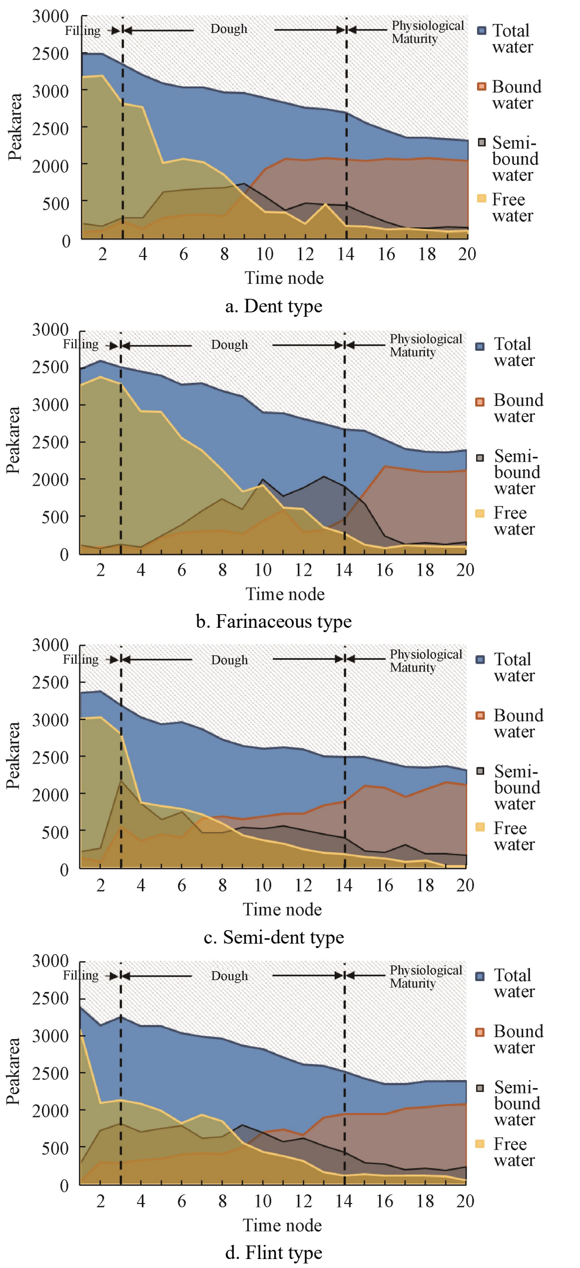


Figure 3 Trends in moisture content in four types of maize

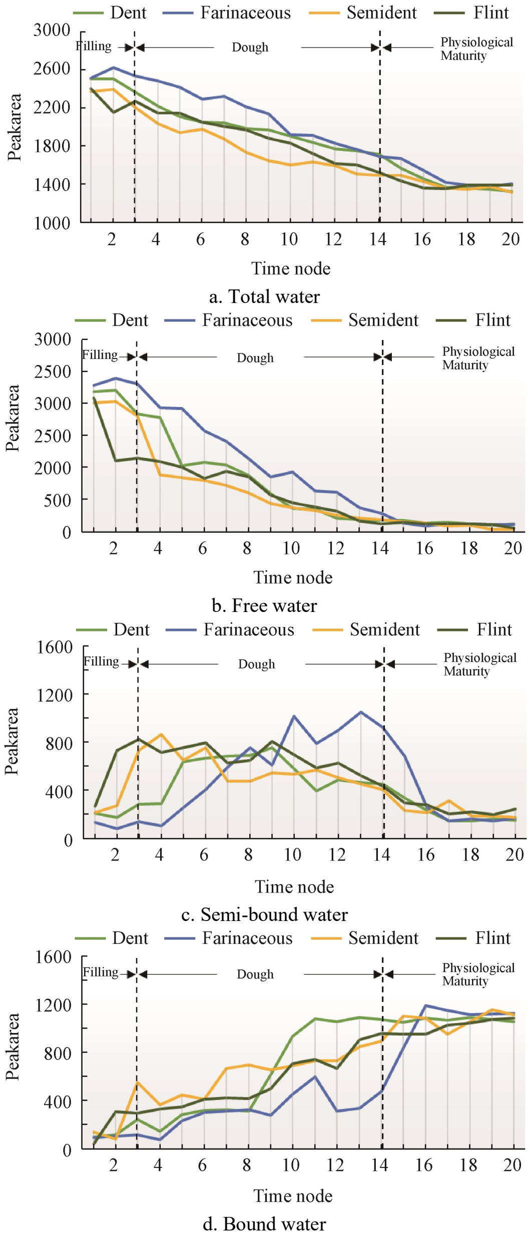
The data shows that the moisture content of different phase states displayed differed among the four kernel types during the period from growth to maturity. The change in total water maintained a steady decline, but peaks appeared at different time nodes. The peak value appeared at node 1 for flint type maize, while the other three types peaked at node 2. The total water content of the farinaceous type was always higher than that of the other three types. The proportion of free water in the maize kernel was very large in the filling stage, usually more than 80% and up to 90%; that is, most of the moisture in the maize kernel is free water. The free water peaked at time node 2 and decreased rapidly in the dough stage except flint type. The proportion of reduced free water accounted for 70%-80% of the total free water. The proportion of free water was highest in the farinaceous type, reaching 88%, and lowest in the semi-dent type. With the continuous growth of maize kernel volume and the continuous deposition of starch in endosperm cells, the proportion of free water decreased very slowly and finally remained stable in the physiological maturity stage. The proportion of semi-bound and bound water increased continuously when entering the dough stage. Semi-bound water increased rapidly in the early dough stage, remained relatively stable for about 20 d, and then decreased at the end of the dough stage and the early physiological maturity stage. The semi-bound water peaked in the middle and late dough stage in the farinaceous type, and the early dough stage in the other three types. The bound water in the four maize types rose slowly during kernel growth and entered a stable state at the late dough stage in all except the farinaceous types. The dent type showed a rapid rise in the middle of the dough stage, while the farinaceous type showed a rapid rise in the early physiological maturity stage, and the other two types showed a steady rise.

The moisture content of each component of the four types changed significantly in the filling and dough stages. After entering the physiological maturity stage, the grain begins to dehydrate, and the contents of free water and semi-bound water are low. This reduced respiratory metabolism and nutrient consumption, and the moisture content of each component basically maintain a relatively stable state.

3.1.2 Analysis of moisture content within each phase state

The different phase states of moisture play different roles in the growth of maize kernels. It is helpful to understand the formation and dehydration mechanisms of maize by exploring the patterns of variation of moisture in the different phase states and the differences among different kernel types. The trends and differences in relative content and phase state among the different kernel types could be intuitively understood as shown in Figure 4.

The total water content of the four types decreased during the entire growth process. The total water content of the farinaceous type was slightly higher than that of the other three types, and in the semi-dent, it was slightly lower. However, the total water content of the four types reached the same level at node 18. The trend in free water was similar to that of total water. The three types apart from the farinaceous type showed a minimum level at node 13, and all four types reached the same level at node 15. The change in semi-bound water rose to a peak, stabilized, and then decreased. The flint type was the first to show a rapid upward trend and stayed at a peak (at a peak area of 800) for the longest time. The farinaceous and dent types began to show an upward trend at node 4, which was faster than the farinaceous type, but the total amount was lower than that of the farinaceous type. The three types apart from farinaceous began to decline at node 12. The bound water increased during the growth of the maize kernel. In the late dough state, the bound water content of the four types showed distinct differences as the dent type had the highest bound water content, and the farinaceous type had the lowest. The differences disappeared by node 18.



a. Total water

b. Free water

c. Semi-bound water

d. Bound water

Figure 4 Comparison chart of relative content change of different types of the same water phase state

3.1.3 Proportions of different phase states

The analysis of the relaxation inversion spectrum data showed that the proportion of moisture in different phase states had a regular trend among varieties, and there were differences among selected types at each stage of maize kernel growth and development.

At the same time node, the relative contents of different phase states of moisture differed among different types of kernels, as shown in Figure 5. At the beginning of the filling stage, the proportion of free water was over 80%; in the farinaceous type, it reached 91.44%. At the end of the filling stage, the proportion of free water decreased significantly in the semi-dent and flint types, from 85% to 50%, while the dent type reached the same proportion nearly 10 d later than the flint type, and the farinaceous type about 15 d later than flint type. The proportion of free water in the semi-dent and flint types decreased rapidly at the end of the filling stage and the early dough stage, while that in the dent and farinaceous types decreased rapidly in the middle of the dough stage. The results showed that the conversion of free water to semi-bound water and bound water was later in the dent and farinaceous types than that in the semi-dent and flint types, indicating that the vigorous metabolism period was longer in the dent and farinaceous types. At physiological maturity, in all four types, the proportion of free water entered a relatively stable period and decreased from approximately 10% to approximately 7%.

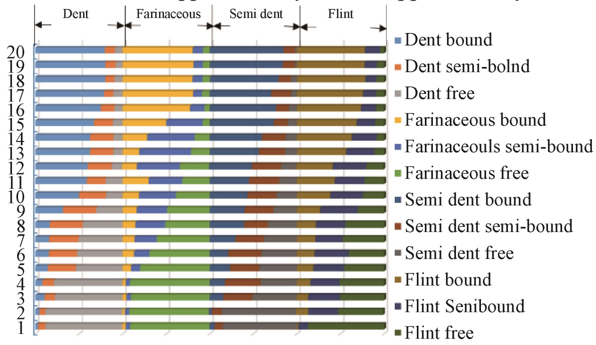


Figure 5 Relative contents of different types of moisture

The proportion of semi-bound water in the farinaceous type increased slowly during the filling and dough stages, while the other three types had similar growth rates. The proportion of semi-bound water in the dent, semi-dent, and flint types began to increase from approximately 15% in the early dough stage to a broad peak, of about 40%, in the middle of the dough stage that was maintained for 12-18 d until harvesting. The farinaceous type had the highest proportion of semi-bound water among all four types, reaching 59% at the end of the dough stage, while the semi-dent type had the lowest proportion of semi-bound water, which only reached 34.8%.

The proportion of bound water in all four types rose during the growth period, from about 3% at the beginning to over 80% at harvest. In the filling stage, except for the farinaceous type, the proportion of bound water increased from 3% to 13%. In the farinaceous type, there was no obvious change with sluggish growth from 3.65% to 4.22%. The proportion of bound water increased most in the dough stage. In the dent type, it increased from 10.23% to 62.61%; in the farinaceous type, it increased from 3.65% to 19.06%; in the semi-dent type from 15.64% to 56.08%; and in the flint type, it increased from 13.00% to 56.56%. Thus, the proportion of bound water in the three types, excluding the farinaceous type, increased by more than 40%. At the physiological maturity stage, the proportion of bound water in the farinaceous type increased substantially, from 28.18% to 80.38%, while in the other three types, it increased by approximately 20%.

3.1.4 Multiple comparisons of the content of different moisture phase states in different types of kernels in the same period

According to the ANOVA, there were significant differences among the contents of the different moisture phase states among the four types of maize kernels at key time points. Five representative time nodes were selected from 12 times nodes for analysis, namely node 1 in the filling stage, nodes 6 and 9 in the dough stage, and nodes 15 and 19 in the stage of physiological maturity.

At the filling stage for node 1, the total water contents of the dent and farinaceous types were significantly higher than that of the flint type (p<0.01), but there were no significant differences among the dent, farinaceous and semi-dent types (p>0.05), nor between the flint and semi-dent types (p>0.05). The free water content of the farinaceous type was significantly higher than those of the semi-dent and flint types (p<0.01), but there was no significant difference between the farinaceous and dent types (p>0.05). There was an extremely significant difference between the dent and flint types (p<0.01), but no significant difference between the dent and semi-dent types (p>0.05). There was no significant difference between the semi-dent and flint types (p>0.05). The semi-bound water content in the flint type was significantly higher than in the farinaceous and dent types (p<0.01), but there was no significant difference with the semi-dent type (p>0.05). There was no significant difference in the semi-bound water content between the semi-dent and dent types (p>0.05), but they were significantly higher than in the farinaceous type (p<0.01). There was no significant difference in the bound water content among the different kernel types.

Table 3 Multiple comparison of relative contents of different water types

Time node	Water type	Dent type Liaodan1281	Farinaceous type Liaodan145	Semi-dent type Xianyu335	Flint type Liaodan502
1	Total water	2483.5856±28.6230^{A}	2486.6806±32.9708^{A}	2435.1175±39.1074^{AB}	2380.1411±35.4476^{B}
	Free water	2185.6541±22.1832^{AB}	2265.4280±72.6977^{A}	2139.7473±48.6714^{BC}	2076.7305±45.1937^{C}
	Semi-bound water	213.7125±21.5474^{B}	130.6853±14.1618^{C}	246.7840±20.9998^{AB}	268.3701±35.3982^{A}
	Bound water	78.1294±62.4966^{A}	90.5673±33.6427^{A}	49.5231±34.1937^{A}	37.8530±30.5162^{A}
6	Total water	2032.7636±21.2984^{B}	2273.8258±27.3388^{A}	1958.8104±26.3521^{C}	2032.6609±26.7066^{B}
	Free water	1075.5164±26.4734^{C}	1568.8745±28.1270^{A}	796.2081±86.7865^{B}	828.8039±10.8970^{B}
	Semi-bound water	665.2029±49.8341^{B}	405.4466±21.4490^{C}	750.0368±104.5771^{AB}	795.2691±30.9715^{A}
	Bound water	319.6423±36.0284^{A}	299.5046±24.9016^{A}	412.5589±117.6911^{A}	408.5878±36.3187^{A}
9	Total water	1956.8721±17.0842^{D}	2119.0637±28.7703^{A}	1768.5539±36.5920^{B}	1865.0809±22.4923^{C}
	Free water	592.2446±5.8243^{B}	850.6023±36.3616^{A}	473.3215±25.1535^{C}	566.9696±28.7639^{B}
	Semi-bound water	750.2465±41.5023^{AB}	605.7784±37.9747^{C}	702.7147±59.8301^{B}	801.5650±36.3149^{A}
	Bound water	614.3810±32.0263^{A}	276.7372±13.1820^{C}	592.5176±54.1491^{AB}	496.5462±80.7222^{B}
15	Total water	1556.9825±13.4146^{B}	1658.7631±33.9246^{A}	1582.2798±26.2822^{B}	1458.4956±38.7561^{C}
	Free water	174.4212±42.4535^{B}	291.7259±18.7282^{A}	152.3164±4.9863^{B}	142.2549±26.6533^{B}
	Semi-bound water	336.8110±21.0770^{B}	700.2637±62.0568^{A}	387.5811±20.1989^{B}	345.3291±10.5102^{B}
	Bound water	1045.8550±35.5849^{A}	666.7734±37.1646^{C}	940.5777±44.9954^{B}	966.1821±37.8540^{B}
19	Total water	1338.8830±92.7374^{B}	1469.8361±39.7063^{A}	1368.4466±1.4888^{B}	1383.6611±17.3047^{AB}
	Free water	104.6411±18.6052^{A}	119.6166±33.1920^{A}	80.3588±9.9904^{A}	115.5265±14.6868^{A}
	Semi-bound water	163.0321±34.0289^{A}	156.2162±10.4528^{A}	173.972±29.1671^{A}	196.0361±15.4839^{A}
	Bound water	1071.2142±49.4769^{B}	1195.3334±1.4436^{A}	1114.1149±23.4901^{B}	1072.0984±10.9121^{B}

Note: ABC: p<0.01

At time node 9, which was in the middle of the dough stage, there was an extremely significant difference in total water content among the four types, and the total water content in the farinaceous type was significantly higher than in the other three types. The relative content of free water in the farinaceous type was also significantly higher than in the other three types. The relative contents of free water in the dent and flint types were both significantly higher than those in the semi-dent type, but there was no significant difference between the free water content in dent and flint. The semi-bound water contents of the dent and flint types were significantly higher than those of the other two types, but there was no significant difference between the semi-dent type and dent type, and there was no significant difference between the dent and flint types. The bound water content of the dent and farinaceous types were significantly higher than those of the other two types, but there was no significant difference between them. The bound water content of the dent and flint types was significantly higher than the farinaceous type, and there was no significant difference between them.

At time node 19, which is the end of the physiological maturity stage, the relative content of total water in the farinaceous type was significantly higher than that in the dent and semi-dent types (p<0.01), where no significant difference was found between that in the farinaceous and flint types (p>0.05). There was no significant difference among dent, semi-dent, and flint types (p>0.05). The relative content of bound water in the farinaceous type was significantly higher than that in the other three types (p<0.01), but no significant difference among the dent, semi-dent, and flint types (p>0.05). No significant difference in either the free water content or the semi-bound water content was found among the different kernel types.

3.2 Comparisons of changes in water content between different varieties of the same kernel type during the growth period

Significant differences in the contents and changes in different moisture phase states in the four kernel types were described in Section 2.1 of the study. To determine whether to investigate further, a similar analysis was carried out investigating the effects of different varieties of maize kernels within the same kernel type.

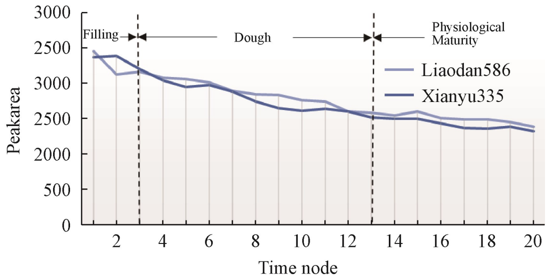
The semi-dent type is derived from hybridization between the flint and dent types, whose plant type and growth characteristics are similar to those of the dent type. The powdery endosperm at the top of its kernel is less than that in the kernel of the dent type but more than that in the flint type, and the quality is better than that of the dent type (which is widely cultivated. In this study, Xianyu335 and Liaodan586 were selected as the research subjects. Both Xianyu335 and Liaodan586 are maize varieties of the semi-dent type and account for a large proportion of the total planted area of maize in China.

3.2.1 Analysis of the changes in relative content of the different phase states

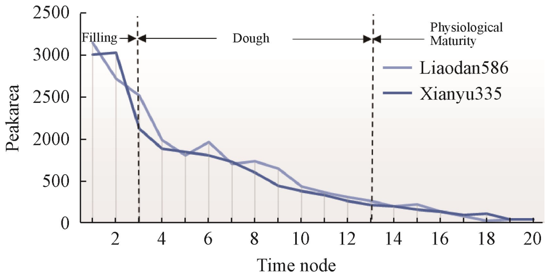
The change rules in total water, free water, semi-bound water, and bound water of Liaodan586 and Xianyu335 were nearly the same (Figure 6). During the entire growth process, the pattern and differences of change in total water content were consistent. There was some difference in the relative content of semi-bound water between the two varieties, but the overall trend was the rapid growth in the filling stage and the slow decline in the dough stage and physiological maturity stage.

3.2.2 Multiple comparisons of contents of different water types during the same period

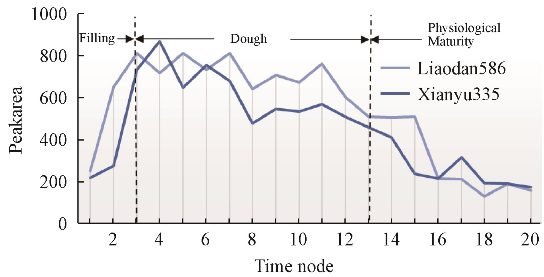
Analysis of variance of total water, free water, semi-bound water, and bound water comparing Liaodan586 and Xianyu335 showed that there was no significant difference in the relative contents of total water (p=0.513>0.05), free water (p=0.477>0.05), semi-bound water (p=0.223>0.05), nor bound water (p=0.975>0.05). The changing trends of total water, free water, and bound water with time were highly correlated.



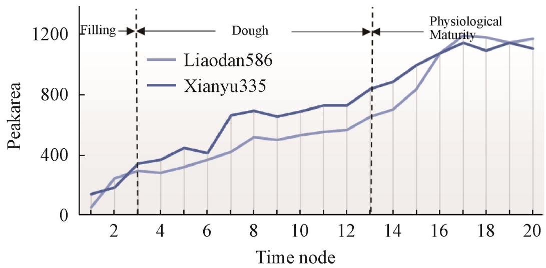
a. Total water



b. Free water



c. Semi-bound water



d. Bound water

Figure 6 Comparison of relative change in contents of phase states of water between two varieties of semi-dent maize

3.3 Correlations between variation in relative content of different moisture phase states and dehydration rate

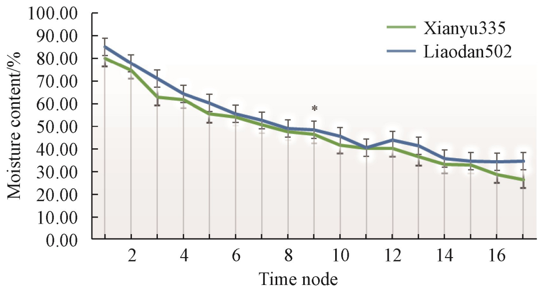
The moisture content is the index of moisture content in the maize kernel. The moisture content of maize kernel at harvest is determined by the moisture content of the kernel at physiological maturity and the dehydration rate of the kernel after physiological maturity. Therefore, the dehydration rate of the kernel depends on the moisture content of maize kernel at harvest[31-34], such as the effects of shading and cultivation methods[35], the contribution of agronomic traits such as bract, stem diameter, and ear diameter[36], genetic characteristics, and QTL mapping[37,38]. However, these studies are based on the average kernel moisture content, ignoring the fact that different phase states of moisture play different roles in metabolism.

The dehydration rate (%/d) refers to the extent of the decrease in the moisture content in the kernels. Currently, the index in Equation (3) was introduced to express the dehydration intensity of the kernel.

$$\text{Dehydration rate} = \frac{\text{Previous kernel moisture content(\%)} - \text{Last time kernel moisture content(\%)}}{\text{Days between sampling (d)}} \quad (3)$$

3.3.1 Analysis of the variations in moisture content and dehydration rate among different kernel types

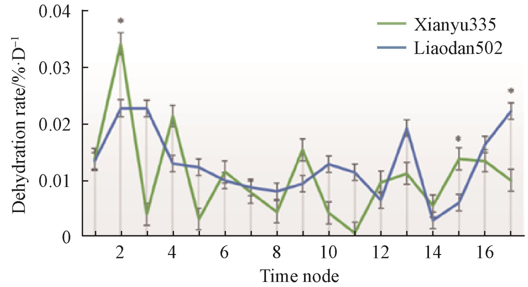
Taking the semi-dent and flint types as examples, the correlations between moisture content and dehydration rate in the dough and physiological maturity stages were analyzed among the different kernel types. The results are shown in Figures 7 and 8, respectively.



Time node	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Xianyu335	0.0012	0.0070	0.0057	0.0030	0.0044	0.0104	0.0058	0.0037	0.0083	0.0052	0.0041	0.0067	0.0039	0.0025	0.0087	0.0025	0.0132
Liaodan502	0.0039	0.0019	0.0040	0.0038	0.0099	0.0045	0.0038	0.004	0.0066	0.0074	0.0098	0.0035	0.0073	0.0015	0.0021	0.0011	0.0220

Note: *: p<0.05.

Figure 7 Trend in moisture rate



Time node	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Xianyu335	0.0232	0.0395	0.0235	0.0113	0.0204	0.0106	0.0026	0.0185	0.0163	0.0226	0.0215	0.0401	0.0203	0.0228	0.0191	0.0259	0.0257
Liaodan502	0.0085	0.0154	0.0139	0.0238	0.0222	0.0387	0.0217	0.0529	0.0061	0.0306	0.0223	0.0290	0.0368	0.0211	0.0317	0.0234	0.0371

Note: * p<0.05.

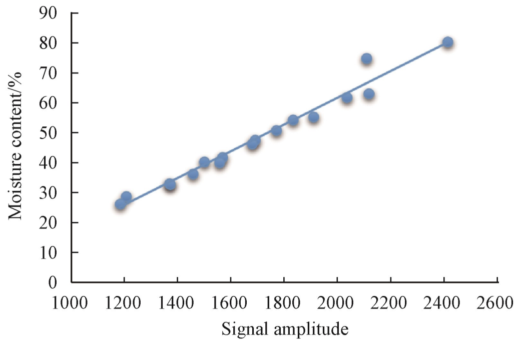
Figure 8 ANOVA of dehydration rate

The kernel moisture content of Liaodan502 and Xianyu335 declined following the filling stage, from 80% to approximately 30% (Figure 7). The moisture content of the flint type was higher than that of the semi-dent type, but the difference was not significant during the entire growth period. According to Figure 8, the dehydration rate of the semi-dent type fluctuated greatly during the filling period and reached the peak level during the entire growth period. The flint type first rose, peaked, and then decreased. At the dough stage, the dehydration rate of the two types remained relatively stable, and decrease to 0.01%/d. Upon entering the physiological maturity stage, the flint type showed a great fluctuation, while the semi-dent type was relatively stable. The results show that the dehydration rates of different kernel types differed in the physiological maturity period, and the flint type had a better rate than the semi-dent type. The dehydration rate of the semi-dent type was relatively stable after the dough stage, which can highly predict the moisture content and the reasonable harvest time. The dehydration rate of the flint type changed greatly in the physiological maturity stage, and it increased rapidly at the end to rapidly reduce the moisture content.

3.3.2 Correlation between total signal amplitude of LF-NMR and moisture content in different kernel types

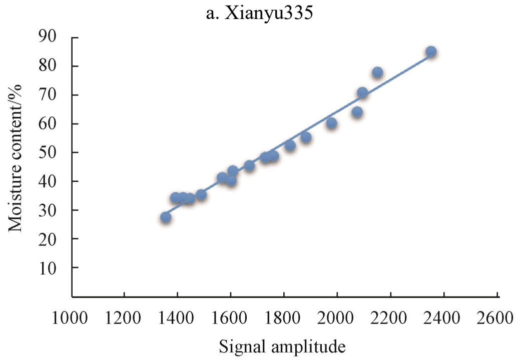
Because the amplitude of the NMR signal is directly proportional to the density of hydrogen protons in moisture, the correlation between the total signal amplitude of the NMR relaxation spectrum and the value of kernel moisture content was analyzed to explore the relationship between them.

Taking Xianyu335 and Liaodan502 as representatives, the total peak area of the LF-NMR inversion spectrum was fitted with maize kernel moisture content, and a correlation analysis between the total signal amplitude of LF-NMR and maize kernel moisture content was carried out. As shown in Figure 9, a significant correlation between the moisture content of the kernel and the total signal amplitude of LF-NMR was developed to obtain the moisture content of the samples according to the total signal amplitude and linear equation.



Note: R^2=0.9756, y=0.0443x–26.817.

a. Xianyu335



Note: R^2 = 0.9782, y=0.0006x–0.4549

b. Liaodan502

Figure 9 Correlation between total signal amplitude and moisture content

3.3.3 Effect of moisture content in different moisture phase states on dehydration rate

Kernel dehydration can be divided into two stages the first stage of the dehydration of the kernel development, and the second stage of the dehydration of the kernel after maturity[11]. The dehydration rate in the second stage dominates the moisture content of maize kernels at harvest. The correlation between the content of different water phase states and the dehydration rate was analyzed as listed in Table 4.

Table 4 Correlation between relative content of different moisture phase states and dehydration rate

Type	Free water	Semi-bound water	Bound water
Xianyu335	−0.956*	−0.971*	0.844
Liaodan502	−0.959*	−0.955*	0.890

Note: *p<0.05

The results in Table 4 list that the relative contents of free water and semi-bound water were significantly negatively correlated with the dehydration rate in the two kernel types, while the relative content of bound water was positively correlated with the dehydration rate. The correlation coefficients of free water and semi-bound water were greater than 0.9 (Pearson correlation coefficient $|r|$≥0.9), indicating a high correlation with the dehydration rate. The r of bound water was between 0.8 and 0.9 (0.9≥$|r|$≥0.8), indicating a correlation between the relative contents of bound water and dehydration rate; but it was not significant.

4 Discussion

The improvements to the yield and quality of maize would be of great significance for ensuring food security, as would the transformation of maize production modes including mechanization through the whole process[36]. The key to this is to cultivate maize varieties with fast dehydration and low moisture content. This study on the patterns of moisture content in the kernels can provide a reference for breeding excellent maize including varieties for mechanical harvest.

4.1 Changes of moisture content in different phase states during maize growth

The moisture content of maize kernels is maintained at a high level in the middle filling stage and begins to enter a dehydration stage at the end of filling while reducing respiratory metabolism and nutrient consumption[17]. The patterns of changes in the total amplitude of the LF-NMR T_2 inversion spectrum that were observed in this study were consistent with the results of previous studies. The total water content of kernels (the total amplitude of LF-NMR T_2 inversion spectrum) decreased from the later filling stage to the end of physiological maturity. Traditional methods of moisture measurement display differences in kernel moisture content among different varieties[17]. This study found differences among different types, and among varieties of the same type, by analyzing the changes in kernel moisture content in different phase states. The differences among types were more significant than those among varieties.

The changes in free water content were consistent with the changes in total water as reduction at the later filling stage, and the reduction rate reached a peak at the early dough stage. With the decomposition, transportation, and synthesis of matter in the dough stage of the maize kernel, the content of free water decreased and gradually converted into semi-bound water and bound water and then evaporated with the transpiration of plants. The content of semi-bound water first increased and then decreased during the entire growth period and was maintained at a high level during the dough stage. The dough stage is the key period for starch deposition and active substance conversion. The semi-bound water in the embryo and endosperm is gradually replaced by storage substance[39] and converted to bound water.

The farinaceous type had the highest content of semi-bound water, and it was also the last to enter the stable stage. At the end of physiological maturity, with the dry matter accumulation of kernels stops, the exchange of moisture and nutrients between kernels and parent plants slows; the metabolic functions approach a "static state"[11]. It was observed that the contents of free water, semi-bound water, and bound water gradually entered a stable state.

4.2 Relationship between the proportions of moisture content in the different phase states and kernel types

Based on the analysis of the proportions of different water types, the trends observed in the proportions of the three moisture phase states were similar in the four kernel types with obvious differences. No significant difference between the types was found at the moisture content of a certain phase state of less than 10%. The result indicated no significant difference between the bound water at the early filling stage and the free water at the later physiological maturity stage. The differences were mainly observed in the dough stage. The dough stage is the key period for the deposition of starch, proteins, and other storage substances.

Water molecules are closely combined with starch, protein, fat, and other macromolecules, and the activities of various enzymes in the endosperm are reduced, resulting in an increase in the proportion of bound water and semi-bound water, and a decrease in free water[17].

The proportions of different types of nutrient substances differed (Table 1), resulting in differences in the proportion of bound water, semi-bound water, and free water. The proportion of free water decreased from more than 80% to approximately 7% during growth. The proportion of semi-bound water achieved peak level at the dough stage, reaching 40%. The proportion of bound water increased from approximately 3% at the beginning to more than 80% at harvest. Therefore, most of the moisture in the early development stage of maize was free water, while most of the moisture in the kernel at harvest was bound water, which is consistent with the previous investigation of the proportions of moisture in the different phase states during the growth[17,28].

Among the four types of maize, the farinaceous type had the highest total moisture content, the flint type had the lowest, and all the moisture contents tended to be equal by the end of physiological maturity. The proportion of free water dropped to less than 50% in the semi-dent type earlier than in the others types. Therefore, the proportion of bound water and semi-bound water was dominant, indicating that this type entered the storage material deposition stage earlier. The farinaceous type differed (significantly in most cases) from the other three types. The change in the proportion of free water showed that this type had a more active process of cell division and differentiation than the other types, and these processes occurred over a longer period[39]. The proportion of bound water was the last to reach a stable state, but the kernel was able to dry and dehydrate at physiological maturity after the accumulation of dry matter stopped. The proportion of free water rapidly decreased to its minimum, which may be related to the fact that the number of bracts is greater than in the other three types. The moisture content of maize kernels is hereditary[37,38]. Consistently, this study found that most of the data indexes of the semi-dent type did not differ significantly from those of the dent or flint types at the same time node. The reason may be that the semi-dent type is a hybrid of dent and flint types.

4.3 Relationship between dehydration rate and moisture content in different phase states

The dehydration of the maize kernel is divided into two stages, developmental dehydration and drying dehydration, when the phase state of moisture also changes dynamically during the dehydration process[11]. In this study (as shown in Figure 9), it was found that the kernel moisture content had a significant linear relationship with the total signal amplitude, which is consistent with previous studies[23,28]. The main mechanism for the drying and dehydration of kernels is the water evaporation on the surface of kernels. The semi-bound water and free water are not tightly bound with starch, protein, and other macromolecules in kernels are more likely to be lost than bound water. This study showed a correlation between the dehydration rate and the phase state content of moisture in the kernels at physiological maturity, and the correlation between free water and semi-bound water was higher than that between free water and bound water, which is consistent with the results of previous research. In addition, there is a dispute about the dehydration rates of the dent and flint types[36]. The trends of semi-bound and free water contents observed in the two types in this study show that the dent type has a great ability to dehydrate than that of the flint type.

5 Conclusions

Low-field nuclear magnetic resonance (LF-NMR) was used to study the changes in the moisture storage state of four types of maize kernels during the growth process. The results showed that there were three phase states, namely free water, semi-bound water, and bound water, with differing trends over time. The trends in free water and total water content were nearly the same, reaching a peak at the end of the filling stage; the semi-bound water generally increased to a peak at the dough stage that then lasted for more than 10 d; the bound water initially rose, peaked at the end of physiological maturity and stabilized. It was the type of maize, not the maize variety, that was the key factor for differences in the phase state of the moisture in maize kernels during the growth period. The metabolic intensity of kernels at different stages can be inherited by hybridization. This method only found that there were differences in moisture phase states content of different maize types in different periods, but the fundamental reasons need to be further studied.

There was a high correlation between the phase states and the dehydration rate, especially between the contents of semi-bound water and free water and the rates of kernel drying and dehydration. The farinaceous type had the highest moisture content among the four types, which was also the type with the strongest dehydration ability at physiological maturity. The semi-dent type had the least free water content at physiological maturity, which can reduce the probability of mildew during storage. The flint type had a higher relative content of semi-bound water at harvest, and semi-bound water content could be reduced by extending the harvest duration to reach a lower level. LF-NMR technology can provide in depth analysis of the changes in the phase state and content of moisture in the kernels of maize, and offers an important means for screening and improving germplasm.

Acknowledgements

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