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DETECTION METHODS OF RICE SEED MOISTURE UNDER DIFFERENT ENVIRONMENTS

Métodos de detección de humedad en semillas de arroz bajo diferentes ambientes

Yan LI[1], Gang CHE[2]*, Lin WAN[2], Chang LIU[3]

[1]College of Agriculture and Hydraulic Engineering, Suihua University, Suihua 152000, China
[2]College of Engineering, Heilongjiang Bayi Agricultural University, Daqing 163319, China.
[3]Faculty of Western Languages, Harbin Normal University, Harbin 150000, China

*Author for correspondence: Chegang1972@126.com

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Key words: Rice seeds soaking technology, water absorption, soaking temperature.

ABSTRACT

In order to study the change of water content of rice seeds during seed soaking, a method for detecting seed water content in different environments is proposed in this paper. According to the multi-component characteristics of relaxation time, the change and flow process of water in different phases of rice seeds were analyzed by the relaxation spectrum, and the regression equation between the total area of the relaxation spectrum and the water absorption rate of rice seeds was determined. The results showed that the water uptake of rice seeds could be reasonably estimated by the total area of the relaxation spectrum, and that the combined water and total water content increased with the increase of soaking time. While the free water changed irregularly, the water uptake of rice seeds was not significantly different between two rice varieties, but it was highly sensitive to soaking temperature. This temperature can reveal the regularity of water content of rice seeds in the soaking process more directly and accurately and provide data support and theoretical basis for searching the best soaking conditions.

Palabras clave: tecnología del remojo de semillas de arroz, absorción de agua, temperatura de remojo.

RESUMEN

Para estudiar el cambio en el contenido de agua de las semillas de arroz durante su remojo, se propone un método para detectarlo en diferentes ambientes. De acuerdo con las características multicomponente del tiempo de relajación, el proceso de cambio el flujo de agua en diferentes fases de las semillas de arroz se analizó por el espectro de relajación, y se determinó la ecuación de regresión entre el área total del espectro de relajación y la tasa de absorción de agua. Los resultados mostraron que la absorción de agua podría ser razonablemente estimada por el área total del espectro de relajación, y que el agua combinada y el contenido total de agua aumentaron con el aumento del tiempo de remojo. Aunque el agua libre cambió de forma irregular, la absorción de agua de las semillas de arroz no fue significativamente diferente entre dos variedades de arroz, pero fue altamente sensible a la temperatura de remojo. Esta temperatura puede revelar la regularidad del contenido de agua de las semillas de arroz en el proceso de remojo de forma más directa y precisa y proporcionar datos de apoyo y base teórica para la búsqueda de las mejores condiciones de remojo.

INTRODUCTION

As one of the most important economic crops, rice is widely used in many fields such as biochemistry, pharmaceutical engineering and so on. The world produced about 400 billion kilograms of rice a year on average in the late 20th century, with an area of about 145 million hectares planted. Ninety-five % of the world's rice produced is eaten as food by humans. The origin of rice is China. The history of rice cultivation in China is 14 000-18 000 years (Saito et al. 2020, Wu et al. 2020). Seeds are one of the most basic means of production in agriculture, but also an irreplaceable way of production. Both the yield of crops and the quality of their products are closely related to the quality of their seeds. After being planted, viable crop seeds are found to have significant advantages in terms of growth and productive potential (Zhao et al. 2020). Therefore, the quality of the seeds is of great importance in the development of agricultural production (Fei et al. 2020, Hu et al. 2020, Haldipur and Srividya 2021). There are many factors that can affect seed viability, such as genetic factors, climatic conditions during seed maturation, planting conditions, seed maturity, mechanical damage, and drying and storage conditions. An important factor closely related to seed vigor is the seed water content, which is directly related to seed quality and economic benefits, and is also an important factor in determining the growth of seedlings (James et al. 2019, Chuong et al. 2020, Guan et al. 2021).

An essential factor of seed life is the amount of seed moisture content. Seed moisture content is expressed as a percentage of the total weight of the seed. It is closely related to seed maturity, optimal harvest time, and preservation method. The water content had a significant effect on seed germination rate and seedling rate (Dhaliwal et al. 2020). Rapid and accurate determination of seed water content is of great significance in agriculture. Seed water content determination is an important step to control seed water differentiation and ensure seed quality (Zuo et al. 2020).

Seed water content is an important physiological index of seeds. The water content of seeds is closely related to many physiological functions of seeds (Yu et al. 2020). Only when the moisture content of the seed reaches 24%, the embryo begins to germinate, and 40% of water content is the best time for seed germination. Dry seeds must absorb a large amount of water in order to reach germination conditions (Cao 2020). In order to promote rapid water uptake, seeds are usually soaked to germinate as soon as possible. Therefore, it is significant to determine the best soaking conditions, such as soaking solution, soaking humidity, and soaking method, and speed up the water absorption rate per unit of time. At present, some scholars have studied this. For example, Lu et al. (2020), studied a water content detection method of *Camellia oleifera* seeds based on hyperspectral spectroscopy. Taking *Camellia oleifera* seeds as the research object, they measured its water content, established a spectral model, preprocessed the spectrum of the seeds, extracted the effective sensitive wavelength through stepwise regression, established a prediction model by using BP neural network and radial basis function (RBF) neural network, and verified the model externally, and selected the optimal prediction model. As well, Zhang et al. (2020) designed a near-infrared rapid quantitative detection method of single corn seed moisture based on random forest (RF). They used a variety of spectral preprocessing methods to eliminate the noise interference caused by particle shape when collecting the spectrum of a single seed. Then principal component analysis (PCA) and denoising automatic encoder (DAE) were utilized to extract the dimensionality reduction features, and a prediction model of single corn seed moisture content based on RF were established. Although the above methods can achieve the prediction of plant water content, they do not take into account different environmental conditions, so there is a certain deviation. In order to accurately know the water situation of rice in different periods and improve the survival rate and quality of rice, this paper studies a rice seed water detection method considering different environmental conditions.

MATERIALS AND METHODS

Experimental equipment

Historical observational data, such as daily maximum temperature in the two full months from July to August 2020, were obtained from 42 local meteorological stations in Liao Ning province, if the daily maximum temperature exceeds 35 ºC for three consecutive days or more, it indicates that rice has been damaged by heat hazard. The Hobo temperature and humidity recorder model U23-002 was used in each weather station area to obtain the values of air temperature and humidity. The parameters of the recorder are shown in **table I**. The instrument was placed in the observatory in each meteorological station area, and a Hobo probe was placed half a meter away from the paddy field to measure the

TABLE I. PARAMETERS OF HOBO TEMPERATURE AND HUMIDITY RECORDER.

Model number	U23-002
Range of temperature measurement	–40ºC-70ºC
Temperature measurement resolution	0.02ºC (25ºC)
Measuring accuracy	± 0.21ºC (0-50ºC)

seed temperature of the paddy field. The ground meteorological observation data of the observatory in all counties and cities of the province from July to August 2018 and the synchronous observation of the paddy field in all regions were obtained by using the recorder (Yang et al. 2020).

Experimental methods

Firstly, some data were randomly selected to be used as the verification data of probability calculation, and the remaining data were used to construct the probability calculation formula (Cervantes-Campero et al. 2020, Tiwari 2020, Wang et al. 2020a). Heat damage probability of paddy field was calculated by temperature at station. To test the probability function of thermal damage we used the chi square test. Seed moisture detection in the process of rice seed soaking evaluates the coincidence between the sample database of high temperature heat injury index and the database fitted by a presupposed distribution function (Bond et al. 2019, Bonanomi et al. 2020, Nitek et al. 2020). The flowchart of the goodness test for the probabilistic fitting of moisture content in seeds subjected to heat injury is shown in **figure 1**.

In order to study the effects of different flooding times and days on the growth of rice seeds, the stage of artificial flooding was in jointing and booting stage of mid-July and heading and flowering stage of mid-August respectively. In the two different periods of flooding, there were 3 days, 5 days and 7 days of continuous flooding respectively in the experimental area, and two repetitions in each experimental treatment. Considering that the rice plant is relatively high and the watered depth is not the key factor, the watered depth was designed to be 30 cm in order to submerge the rice root system (Sanchez-Diaz et al. 2019). During the experiment, special personnel irrigated the area day and night. The experiment was carried out on the medium fertility responsibility field with 114^{o} 01' longitude, 33^{o} 00' latitude, 12 test areas, 10 m^{2} (2 x 5 m) each, 0.5 m width and 0.4 m height of the soil bank around, and anti-seepage membrane embedded in the bank. The test arrangement is shown

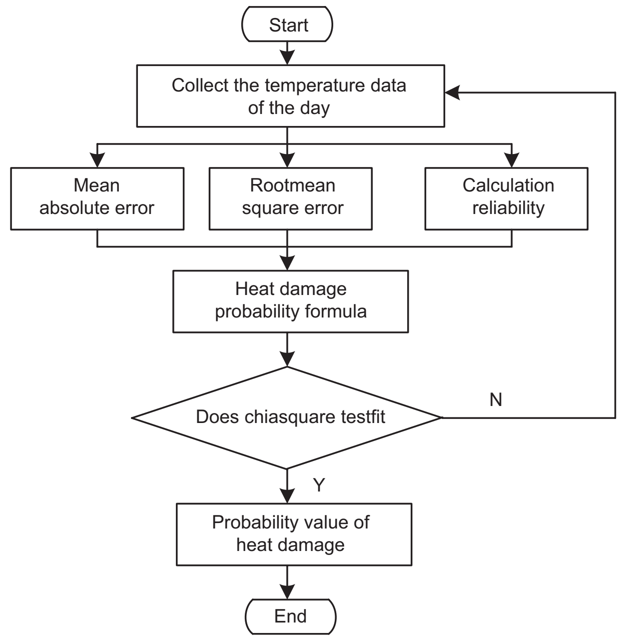

Fig 1. Flow chart of test method for goodness of fitting probabilities of moisture content of seeds with high temperature heat injury.

figure 2. Two control areas with different water storage periods were selected at the edge of the test field (Ma et al. 2019). Each treatment was arranged randomly and repeated twice. There was a rectangle of 10 square meters (2 x 5 m), with an interval of 0.5 m and aisle 1.0 m. Protective rows were set around the area, as shown in **figure 2**.

The experiment was designed as a solar greenhouse with a length of 56 m, a width of 12 m, and a ridge of 3.5 m. The plot area was 14 m2, and 40 plants were planted and repeated thrice. The period of control was April 10 to August 20. The design of the laboratory was a solar greenhouse. The length was 56 m, the width was 12 m, and the ridge was 3.5 m. The greenhouse is leveled after watering. The upper part is 65 cm wide, the lower part is 60 cm wide, and the depth is 25 cm. Solar greenhouse groove bottom and four walls pave 0.1 mm thick double layer film and soil isolation; both sides pressure a layer of the brick can. Rice seeds were cultivated in a greenhouse with a length of 50 m and a width of 7 m (Zhao et al. 2021).

First of all, the seed morphological characteristics were observed. In the process of selecting the best seed of rice seed varieties, the method of directional selection of single plant was adopted. The first flower node was 6-7 knots, and the father parent was B49-5-3-1. One thousand seeds were

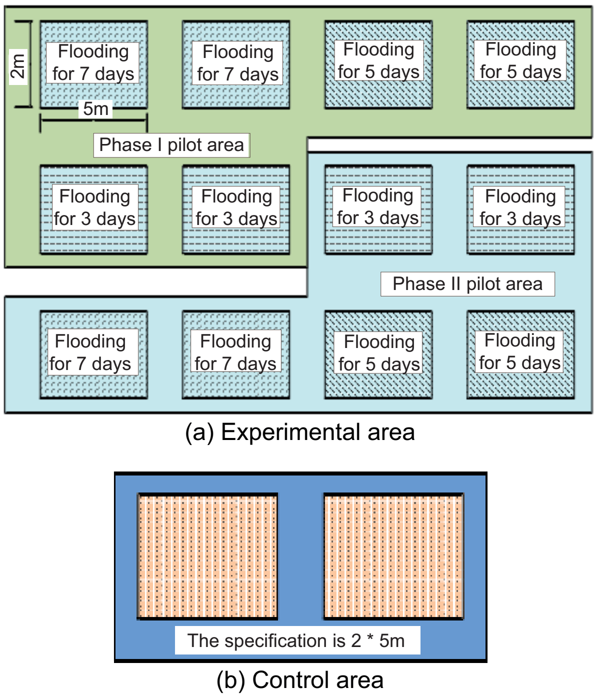

Fig. 2. Experimental area map.

randomly selected. The weight of 1000 seeds was measured by a ten-thousandth electronic balance and repeated for 5 times. 50 seeds were randomly selected and the size of seeds (length and diameter) was accurately determined by a vernier caliper. According to the experimental environment, seed germination experiments at different temperatures were carried out in solar greenhouse with LRH-250-G incubator (produced by Guangdong Medical Instrument Factory). Seeds germination experiments at different temperatures were carried out. The seeds were washed with water before the experiment, and the full seeds were selected by the water separation method. Under the conditions of four temperature gradients of 20ºC, 25ºC, 30ºC, 20-30ºC and periodical illumination (3000lx, 12 h/d), seeds were carried out repeatedly with 50 seeds in each group, and three seeds were carried out repeatedly. The germination medium was a 10 cm dish and the filter paper was 0.5 cm thick. The seeds were fixed in the substrate to avoid proper movement of the seeds during watering. Distilled water was replenished properly according to the condition of dehydration of the culture dish. The germination standard was 0.5 cm, the number of germinated seeds was counted every 4 days, and the experiment ended when no seeds germinated continuously for 20 days.

Ten mature and and full rice seeds were weighed in a TE313S-DS electronic balance; the total weight was 0.251 g as a sample. All samples were randomly assigned to different soaking methods according to the soaking conditions, and 18 parallel samples were prepared according to the serial number of each sample.

Group 1 experiment sample

The sample type was Shen Nong 9816, and the sample number 108 (including 18 parallel test samples for each soaking time). The soaking time ranged from 1 to 7 hours. The soaking method was continuous soaking, the soaking solvent was clear water, and the soaking temperature 18 ± 1 ºC.

Group 2 experiment sample

The samples were Shennong 9816, Qishan Zhan and Xiuzinuo, and the number of samples was 432 (18 parallel test samples for each type and soaking time). The soaking time ranged from 1 to 48 hours. The soaking method was continuous soaking.

Group 3 experiment samples

The samples were Shennong 9816 and Qishan, the soaking solvent clear water, and the soaking temperature 25 ± 1 ºC. The samples were taken out at 6, 12, 18, 24, 30, 36, 42 and 48. After soaking, the sample was removed and drained, the surface moisture was wiped off with absorbent paper. To prevent the moisture from evaporating, it was puted into a 10 mL separate tube for detection.

RESULTS

Under the frequent occurrence of flood and waterlogging disasters

Because the impact of frequent flood disasters on the agricultural production efficiency of rice planting is ultimately reflected by the yield index, we made a specific analysis of variance on the yield index to make the analysis of experimental data more scientific and accurate. The results are shown in **table II**.

From the multiple comparisons of the number of inundation days and the effect of the inundation period on yield in **table II**, we can conclude that the influence degree of inundation days on yield is as follows: inundation days 7, inundation days 5, inundation days 3. The influence degree of the inundation period is larger than that of the heading and booting periods. On the one hand, the purpose of this experiment is to judge the impact of different flood-

TABLE II. EFFECTS OF DIFFERENT FLOODING TIME AND DAYS ON RICE YIELD.

	Source of variation	Output
Days of flooding	Flooded 0d	750.5a
	Flooded 3d	265.66b
	Flooded 5d	150.345c
	Flooded 7d	51.58d
Flooding period	Jointing booting stage	248.15b
	Heading and flowering stage	360.89a

ing indexes on rice production efficiency through a flood disaster simulation experiment, and establish the relationship model between flooding days and rice yield loss rate through the experiment, in order to provide reference for rapid evaluation of the impact of flood disaster on rice production efficiency (Fan et al. 2020). Therefore, according to the experimental data, a simple model of the relationship between the number of inundation days and the loss rate can be made, as shown in **figure 3**.

As shown in **figure 3(a)**, the relationship model between the number of submergence days at jointing and booting stages and the yield loss rate of rice is as follows: $y = 0.2833x^3 - 5.8929x^2 + 41.1132x$. The correlation coefficient is 0.9999. However, due to the limited data, its accuracy needs to be improved through the next experiment, but it can be roughly estimated in the rapid evaluation (Yan et al. 2018).

As shown in **figure 3(b)**, the relationship model between the number of submergence days at heading and flowering stage and the yield loss rate of rice is as follows: $y = 0.4293x^3 - 5.3464x^2 + 29.259x$. The correlation coefficient reached 0.9997, through SPSS software, regression analysis, the equation also passed the significance test of 0.01 level. However, due to the limitation of experimental data, the accuracy of the model needs to be further improved through later experiments (Xu 2012). It can play a role in rapid flood loss assessment.

As shown in **figure 3(c)**, when the influence factor of the submergence period is not considered, a relationship model between submergence days and rice yield loss rate can be established: $y = 0.3563x^3 - 5.6196x^2 + 35.185x$. The correlation coefficient of the equation reached 0.9316, and through regression analysis, it reached the significance test of 0.01 level.

Through the analysis of **figure 4**, it can be seen that the method of this paper has a high fitting degree between the predicted temperature and the actual temperature and can effectively predict the probability of heat damage. The optimal distribution of

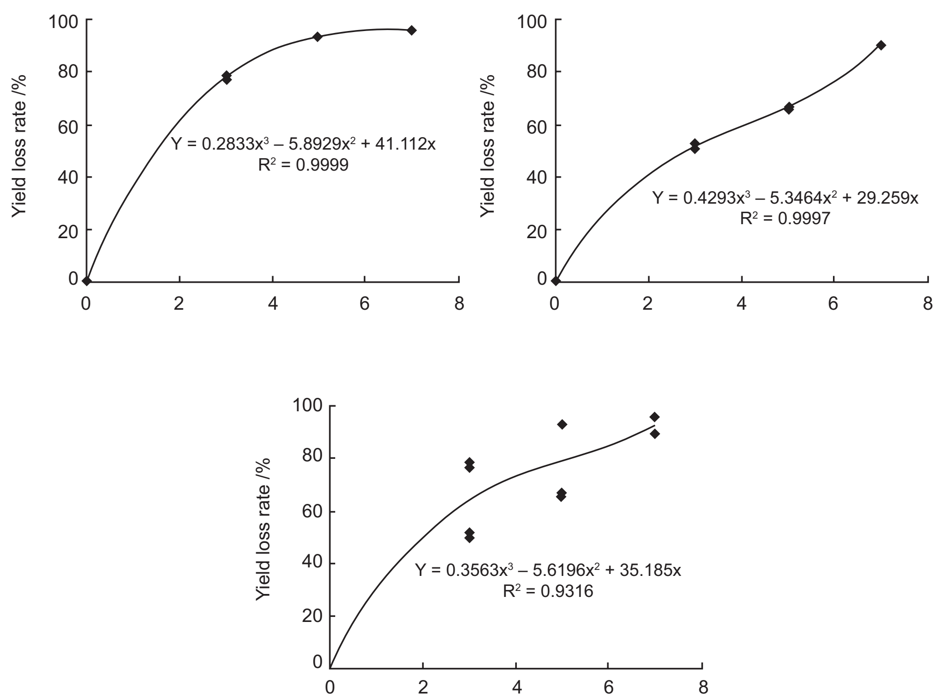

Fig. 3. Relationship between days of submergence and yield loss rate of rice.

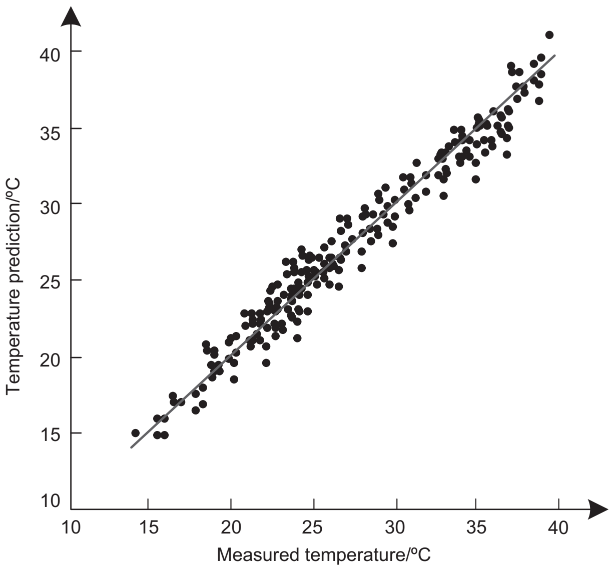

Fig. 4. Fitting results.

heat injury probability of rice is 7 stations of normal distribution model, 5 stations of normal logarithmic distribution model, 6 stations of generalized extreme value distribution model, 5 stations of gamma distribution model, 10 stations of information diffusion model and 9 stations of distribution model (Wang et al. 2020b).

Solar greenhouse conditions

The experimental results show that the influence of illumination on seed germination and are shown in **figure 5**, and as can be seen, the seed germination rate was 33.34% under periodic light and 37.3% under continuous darkness. The relationship between rice seed expansion and rice seed degeneration rate is shown in **table III**. The results in **table III** showed that there was no significant difference between D and E treatments, which showed that light had a significant effect on seed germination.

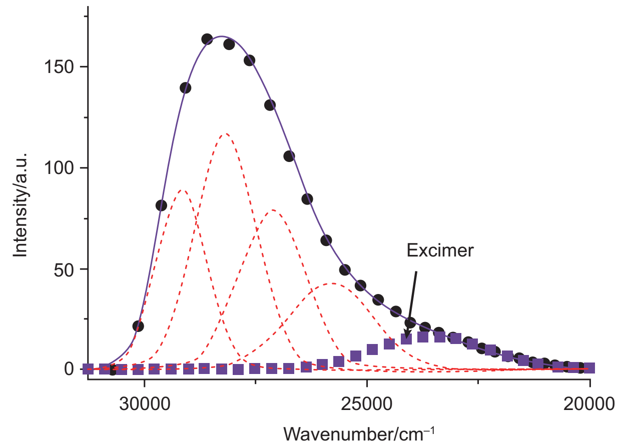

Fig. 5. Curve of influence of illumination on seed germination.

Effect of seed soaking process on different water distribution

The total amplitude of NMR relaxation spectrum is the summation of the peak area corresponding to each peak in T2 relaxation spectrum, which is also the total signal intensity of NMR. The relationship between NMR signal and water content of rice seed sample is very significant. Because NMR signal is directly proportional to the number of ammonia atoms in the sample, the relaxation spectrum reflects the corresponding pure water content of rice seed sample.

Effect on the distribution of combined water moisture

Four samples were selected to analyze the moisture content of rice seed combined with water during soaking. The amplitude of NMR signal of each sample at different soaking time is shown in **table IV**. The variation curve of the signal amplitude A21 of rice seeds during soaking with soaking time, is shown in **figure 6**. From **figure 6**, it can be seen that there are six increasing points in the increase of the bound water of four groups of samples in 24 units time, and

TABLE III. DETERMINATION OF RICE SEED DEVELOPMENT AND RELATIONSHIP BETWEEN RICE SEED DEGENERATION RATE.

Processing combination	Germination expansion of rice seeds/h	Nutrient matrix depletion/L	Utilization ratio/g/L	Rate of stamen degeneration in rice
A	798.23	43	16.364	92.36
B	756.36	43	17.251	94.34
C	769.25	43	16.324	93.25
D	752.14	43	16.021	90.31
E	602.36	43	13.028	93.28

TABLE IV. COMBINED WATER NMR SIGNAL AMPLITUDE.

Sample number	Signal amplitude/AU						
	1 h	2 h	3 h	4 h	5 h	7 h	7 h
A	1619.19	2000.22	2089.27	2269.78	2502.52	2534.22	2609.85
B	1610.79	1962.66	2214.09	2379.96	2563.93	2651.48	2755.13
C	1609.99	1965.87	2195.36	2401.76	2422.40	2615.51	2715.49
D	1581.18	1953.13	2191.92	2397.89	2414.81	2579.11	2725.73

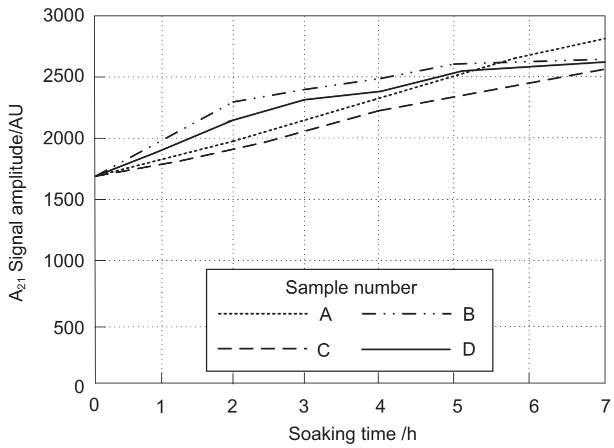

Fig. 6. Variation of signal amplitude of rice seed A21 with soaking time.

the others are all downward trend. With the extension of soaking time, the physiological activity of seeds is enhanced, and the reaction between water and organic matter is promoted. When the bound water reaches a certain degree of saturation, the increasing trend will be slowed down.

Bound water is a kind of stable water, which exists in the chemical substances in rice seeds and has a binding state with material molecules. In the soaking process, the seed coat softens and the air permeability of the seed coat increases. Proteins, starch and carbohydrates stored in seeds will be hydrolyzed to participate in the reaction.

Effect on the distribution of free water moisture

Four samples were selected to analyze the free water status of rice seed during soaking. The amplitude of NMR signal of each sample at different soaking time is shown in **table V**. The variation curve of A22 signal amplitude of free water with soaking time during soaking of rice seeds is shown in **figure 7**. Free water is water that is not adsorbed by colloidal particles or macromolecules in plant cells and can move freely and act as solvent. The signal amplitude of free water changes irregularly during the soaking time of rice seeds in four groups, which is due to the softening of seed coat and the flow of outside water into seed cells.

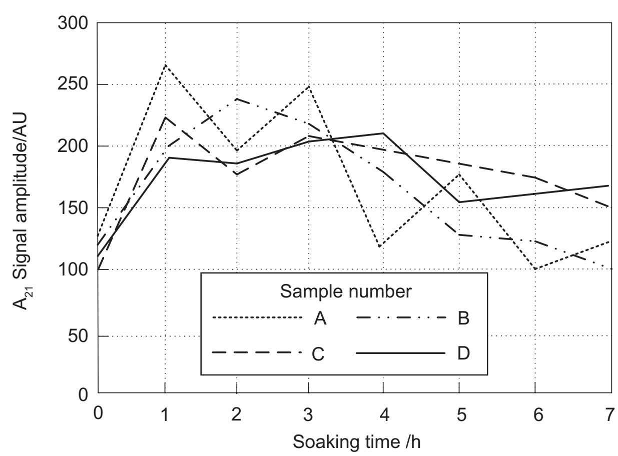

Fig. 7. Variation of signal amplitude of rice seed A22 with soaking time.

TABLE V. FREE WATER NMR SIGNAL AMPLITUDE.

Sample number	Signal amplitude/AU						
	1 h	2 h	3 h	4 h	5 h	7 h	7 h
A	120.44	216.18	174.66	201.35	108.94	144.41	149.25
B	113.65	160.23	196.94	178.36	160.52	136.57	77.36
C	102.97	183.70	163.09	145.32	152.55	83.40	89.31
D	109.80	173.73	168.10	137.04	163.46	145.33	145.25

TABLE VI. NMR SIGNAL AMPLITUDE OF TOTAL WATER.

Sample number	Signal amplitude/AU						
	1 h	2 h	3 h	4 h	5 h	7 h	7 h
A	1739.64	2216.40	2263.93	2482.80	2615.32	2678.63	2790.32
B	1724.44	2122.90	2411.04	2549.26	2724.46	2784.04	2832.50
C	1712.96	2125.57	2358.45	2549.30	2574.95	2698.91	2804.80
D	1690.99	2126.87	2360.03	2534.93	2596.77	2724.44	2884.41

When the proportion of free water increases, the total water content increases, the viscosity of protoplast decreases, the metabolism becomes stronger, the concentration of glucose, fructose and other carbohydrates will increase, and the free water will migrate to the combined state and the concentration of free water will decrease. Free water as a reaction of water solubility, metabolic activity and cell membrane permeability of water in rice seeds is easy to move and loss. Under the influence of soaking time, water moves between free water and combined water, which makes the water phase redistribute, and the content of free water changes irregularly and repeatedly.

Effect on total moisture content

Four samples were selected to analyze the total moisture content of rice seeds during seed soaking. The amplitude of NMR signal of each sample at different soaking time is shown in **table VI**. The change curve of total signal amplitude of rice seeds with soaking time is shown in **Figure 8**. From Figure 8, the total signal amplitude of four groups of rice samples increased as a whole during the soaking time, which indicated that the total water content of rice seeds increased continuously. Free water is a process of continuous flow and migration. Seeds absorb water through the seed coat, and then combine with protein and sugar to convert it into bound water. The soaking time was 7 hours. According to the statistical analysis of the data, the water absorption amplitude of four groups was 60.39%, 64.27%, 63.74% and 70.58%, respectively.

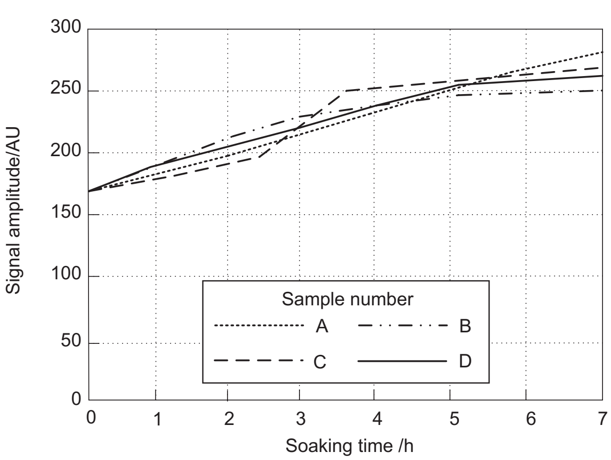

Fig. 8. Variation of signal amplitude of rice seed A with soaking time.

According to the known three kinds of water content, i.e. inner layer water, middle layer water and outer layer water, the proportion of three kinds of water content in the total water content can be calculated, as shown in **table VII**.

TABLE VII. WATER CONTENT RATIO OF DIFFERENT WATER LEVELS.

Soaking time/h	Inner water ratio (%)	Intermediate water ratio (%)	Out water ratio (%)
1	60.89	33.20	5.91
2	43.43	47.85	8.72
3	39.35	50.85	9.83
4	40.21	50.21	9.58
5	36.17	54.02	9.51
6	36.29	53.82	9.89
7	37.04	55.34	10.58

Based on the corresponding data, the curves of the contents of inner layer water, middle layer water, and outer layer water in different layers with soaking time can be obtained, as shown in **figure 9**. The physical properties and metabolic intensity of protoplasts are also different when the proportion of the aqueous phase is different. Compared with bound water and free water, internal water and macromolecule binding are closer, while external water binding is more free.

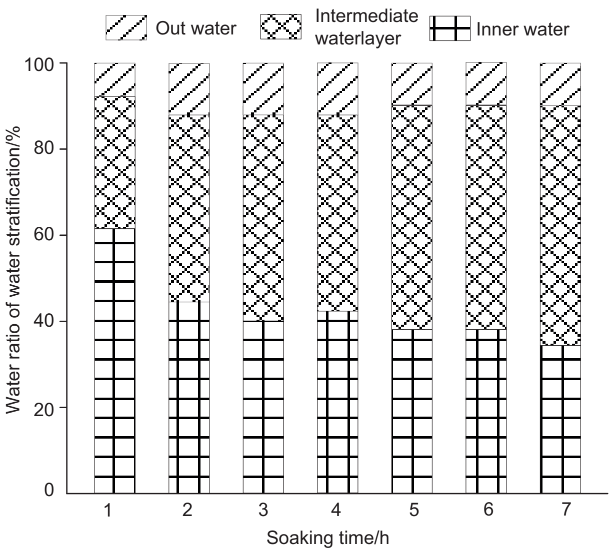

Fig. 9. Curve of moisture content of each layer with soaking time.

DISCUSSION

1) Under the condition of significance level, the regression analysis of rice seeds' water absorption rate and the nuclear magnetic resonance signal's total amplitude showed a relatively consistent regression relationship between them, indicating that the fitting degree of the equation was good. It is considered that there is a real regression relationship between the total amplitude of the rice seed NMR signal and the water absorption rate of the rice seed soaking process, and it is reasonable to use the equation to estimate the water absorption rate of the rice seed soaking process.
2) In the process of continuous seed soaking, the content of combined water and total water increased continuously, while the content of free water changed irregularly and repeatedly. The change of water content can reflect the physical and chemical reaction of rice during seed soaking.
3) The soaking time of 7 h is in the stage of water absorption during seed germination. The water enters into the seed through the softened seed coat and becomes water soluble and permeable water of cell membrane. The water moves freely within the rice seed and is not absorbed by colloid particles or macromolecules in the plant cells and becomes free water with solvent function. With the increase of free water content in total water content, the viscosity of protoplasm decreased and the cell metabolism increased, the concentration of glucose, fructose and other organic carbohydrates increased, and the water migrated to the combined state.

CONCLUSION

In this paper, the above seed moisture detection method was designed and studied, and experiments proved the effectiveness of the design method. However, in order to improve the application effect of the method, we need to pay attention to the following aspects:

1) To ensure the authenticity and reliability of the detection results, it is crucial that we use a larger number of samples to conduct research on moisture detection based on low-field NMR technology.
2) More experimental sample varieties must be selected to carry out water detection research based on low-field nuclear magnetic resonance technology. This research hopes to clarify the water absorption of different rice varieties in the seed soaking process and whether there are similarities in water absorption among different rice varieties.
3) The next research should use a multi-factor experimental design to understand the change in water absorption of rice seeds during the cross-setting of different soaking conditions.

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