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Development of automatic nuclear magnetic resonance screening system for haploid kernels in maize

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Abstract: An automatic screening system for maize haploid kernel identification based on oil xenia effect and nuclear magnetic resonance (NMR) is developed to meet the demand of large-scale application in maize haploid breeding and research. The screening system was comprised of a seed feeding module, a measurement module of oil content and a screening module. The results show that the mean accuracy of this screening system reaches 92.3%. Even with the shorter signal acquisition time, the accuracy does not show a significant decline. The automatic screening system can be used in practical application in rapid selection of maize haploid seeds and can also be used to separate the samples of different oil contents.

Key words: automatic target recognition, screening, intelligent systems, maize, haploid identification, nuclear magnetic resonance
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0 Introduction

Haploids and doubled haploid (DH) lines have become important materials for maize genetic research and breeding[1]. Currently, DH technology based on *in vivo* introduction of maternal haploids has been effectively developed and employed in maize breeding[1-4]. In the near future, a few new inducer lines with even higher haploid induction rate would have been developed[5-7], which should lead to the massive utilization of DH technology and promote the efficiency of maize breeding procedure. A key issue in DH technology on a commercial scale is an efficient screen system to identify haploid kernels from the hybrid kernels crossed with the inducer.

At present, one of the easy, fast haploid identification methods is making use of ‘Navajo’ kernel trait expressed by dominant anthocyanin gene *R*1-*nj*. Seeds with a pigmented endosperm and a non-pigmented embryo are selected as haploids[8-9]. But the shortcoming of this way is that the expression of anthocyanin gene *R*1-*nj* will be affected by donor genotype and environment, which significantly restricts the application of DH technique in large-scale breeding programs[10]. Another accurate, efficient distinction approach is to use high oil xenia effect in maize. There exists large significance between hybrid and haploid seeds generated by high oil inducer, which makes it possible to use oil content in kernel as an indicator to identify the haploid. This method should be able to overcome the shortcomings in the identification method using Navajo marker[11].

Nuclear magnetic resonance (NMR) technique has been routinely used to rapidly measure oil content in sunflower[12-13], maize[14-15], soybean[16], groundnut kernels[17] without destroying seeds. In addition, this

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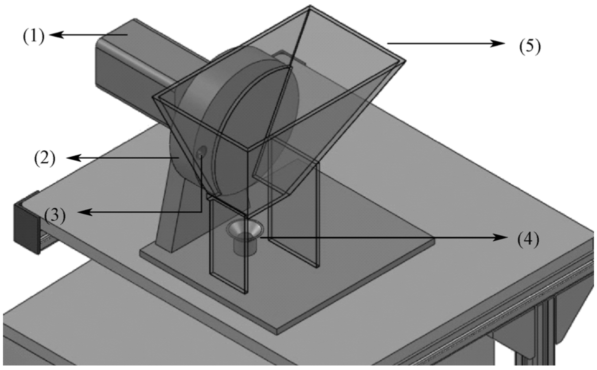
analysis is more precise and reproducible compared with the conventional chemical analysis methods[18]. However, at present oil content of maize seeds still need to be measured via manual work with traditional NMR system, which is time-consuming and requires a lot of labor. This approach cannot meet the demand of modern large-scale maize breeding.

The purpose of this study is to develop a rapid, accurate, and automatic NMR screening system for identification of haploid kernels from *in vivo* introduction of maternal haploid based on oil difference between hybrid and haploid seeds. Furthermore, this automatic screening system can be also used to separate other samples with different oil contents.

1 Seed feeding module

As shown in Fig.1, the seed feeding module consists of a step motor, a subunit of sucking single kernel, a vacuum generator, a pocket for receiving kernel, a hopper. And the subunit of sucking single kernel is composed of a traverser, the pit for absorbing kernel set air suction inlet, which is driven by the step motor associating with the vacuum generator.



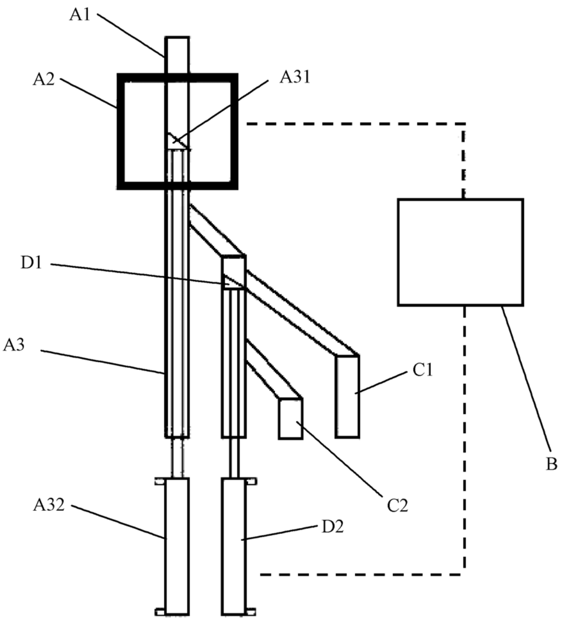
1.Step motor 2.Subunit of sucking single kernel 3.Pit 4.Pocket for receiving kernel 5.Hopper

Fig.1 Schematic diagram of the single seed feeding module

After the step motor is switched on, the traverser rotates periodically following the step motor and the vacuum generator works, which leads to produce negative pressure inside the subunit of sucking single kernel. One of the seeds inside the hopper will be inhaled in the pit for absorbing kernel. While the pit for absorbing kernel loading the single seed arrives over the pocket for receiving kernel, negative pressure is stopped by the vacuum generator. And then this seed falls into the pocket for receiving kernel because of the gravitation.

2 Measurement module of oil content and the screening module

Figure 2 shows four main components of the NMR measurement of oil content and the screening module: a sampling apparatus (consists of a NMR tube, a probe coil and a sample loading subunit), a special purpose computer for NMR, a multichannel sample discharge subunit, an electrical control switch (consists of a switch body and a driving switch set).



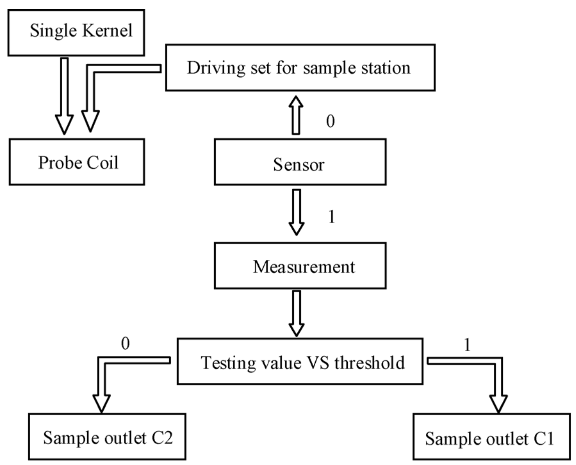
Note: A1, NMR Tube; A2, Probe Coil; A3, Sample Loading Subunit; A31, Sample Station; A32, Driving Set for Sample Station; B, Special Purpose Computer for NMR; C, Multichannel Sample Discharge Subunit; C1&C2, Sample Outlet.; D, Electrical Control Switch; D1, Switch Body; D2, Driving Switch set.

Fig.2 Schematic diagram of the NMR measurement of oil content and the screening module

The probe coil linked with the NMR tube is connected with the special purpose computer for NMR, and an optical path sensor carried by the probe coil can respond sample in order to send out electric signal to the computer. The sample loading subunit consists of driving set for sample station and sample station inside NMR tube and on the center of probe coil. Driving set for sample station is a kind of electronic control and pneumatic driving unit, which is a cylinder controlled by electromagnetic valve connected with computer. Sample station, which is fixed on the top of pushrod of cylinder, can move back and forth following pushrod of cylinder in NMR tube.

Multichannel sample discharge subunit has two sample outlets: one (C1) is connected with NMR tube; the other (C2) is a branch of C1, and is controlled by electrical control switch. Switch body fixed on the top of pushrod of cylinder can move back and forth following pushrod of cylinder in order to switch sample outlets.

The procedure of oil content measurement and kernel screening is shown in Fig.3. The single kernel from the seed feeding module enters the NMR tube and arrives at sample station located in the center of probe coil. The electrical signal ‘1’ generated by the sensor is input into the computer to run the process of measurement of oil content. If the sample is not at the center position of the probe coil, the sensor could not detect the sample. Then electrical signal ‘0’ will be fed back to computer to inform that driving set for sample station drives the sample station to the center position of the probe coil again. After testing, the result of sample oil content will be recorded and saved. If testing value is larger than threshold value, digital signal ‘1’ will be generated, switch body will be put up, and then the sample will be discharged to sample outlet ‘c1’; conversely, if testing value is less than or equal to threshold value, digital signal ‘0’ will be generated, switch body lowered, and the sample discharged to sample outlet ‘c2’.



Note: 0 and 1 stand for different digital signal.

Fig.3 Flow chart of the NMR measurement of oil content and the screening module

3 Verification of screening system

To validate the speed and accuracy of the automatic screening system for haploid maize kernels, high oil induction line CAUHOI[11] with significant oil xenia effect crossed with normal corn inbreds. Table 1 shows that the first filial generation (F_1) seeds of six crosses are screened by the system and the mean accuracy reaches 92.3%.

Furthermore, in order to accelerate the speed of screening but not influence the accuracy of haploid identification, twenty F_1 seeds (15 seeds of Xianyu 335 and 5 seeds of Zheng58/ CAUHOI) randomly selected were measured on 10 replications with acquisition time of 2 and 1 Sec, and the oil contents obtained by the two different acquisition time were compared. Test of significance of difference (Table 2) shows no significant differences between signal acquisition time of 2 and 1 Sec using the screening system. And linear regression fit (Fig.4) indicates that oil content is significantly correlated (r=0.994) with time of 1 and 2 Sec. Based on the above results, from sample feeding to discharge, the average speed of screening using this system will be 4 seconds per kernel.

Table 1 Accuracy of the screening system

Cross	Number of Seeds	Haploids selected	True haploids	Accuracy /%
Zheng58/CAUHOI	561	35	32	91.4
Yu87-1/CAUHOI	586	40	38	95.0
Jing24/CAUHOI	612	46	42	91.3
Huang C/CAUHOI	683	39	36	92.3
B73/CAUHOI	845	48	44	91.6
Mo17/CAUHOI	705	41	38	92.6
Total	3992	249	230	92.3

Note: CAUHOI is an inducer lines with high oil content from National Maize Improvement Centre of China, China Agricultural University

Table 2 Test of statistical significance of the accuracy obtained under different acquisition time length.

Differe-nce	Parameter					t value	df	Sig. (2 tailed)
	mean	standard deviation	standard error	99% confidence interval lower	99% confidence interval upper			
y1-y2	-0.019	0.122	0.027	-0.098	0.059	-0.709	19	0.487

Note：df is degree of freedom; Sig. (2 tailed): the two tailed value probability value; y1 and y2: oil content at NMR signal acquisition time of 1 and 2 second, respectively. The same symbols in the following tables and figures are the same as those in this table.

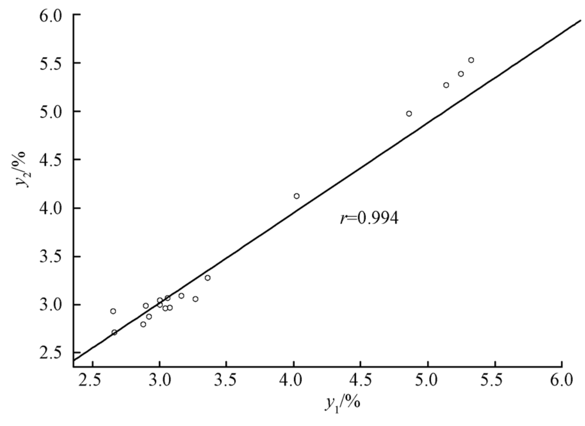


Fig.4 Linear regression fit between NMR signal acquisition time of 2 and 1 Sec

4 Conclusions

In this paper, an automatic NMR screening system for haploid maize kernels based on high oil xenia effect in maize is proposed. The screening system is composed of the seed feeding module, the measurement module of oil content, and the screening module, having the functions of automatic feeding and segregating samples.

Because the visual marker R1-nj for anthocyanin pigmentation is influenced by the genetic background of maternal germplasm and environment, many “false positive” seeds cannot be eliminated by eyes or cameras. The NMR screening system measures the oil content of seeds to identify the haploid seeds. This method does not rely on Navajo marker, which will become a powerful tool for screening haploid maize kernels. The results show that the mean accuracy of this screening system achieves 92.3%. Even with the shorter signal acquisition time, the accuracy does not show a significant decline.

The automatic screening system can be used in practical application in rapid selection of maize haploid seeds and can also be used to separate the different categories of oil content.

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玉米单倍体核磁共振自动分拣系统的开发

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摘要：为满足玉米单倍体育种与研究的大规模应用，本研究利用玉米油份花粉直感效应的生物学特性，开发了玉米孤雌生殖诱导单倍体核磁共振自动识别分拣系统。该系统由单籽粒自动进样模块、玉米油份自动测试模块和自动分拣模块三大模块组成。结果证实该玉米单倍体核磁共振自动分拣系统具有快速（平均速度为 4 秒/粒）、准确（平均为 92.3%）、自动、智能分拣玉米单倍体的优良特性，同时，本系统也可应用于基于油份差异的其他样品的自动智能化筛选。

关键词：自动识别，分拣，智能系统，玉米，单倍体识别，核磁共振