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Identification of maize haploid kernels based on hyperspectral imaging technology

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ABSTRACT

Haploid breeding is a significant technology of maize breeding. Rapid and accurate haploid kernel identification method has great significance to accelerating the efficiency of haploid breeding. At present, detecting genetic markers on the embryo of kernels by machine vision and determining oil content of maize kernels by nuclear magnetic resonance (NMR) are widely used to automatically identify haploid maize kernel. However, the machine vision method can only identify the haploid through embryo side of kernels, and the NMR method cannot distinguish them when haploid and diploid have the overlap oil content. The study was aimed exploring a rapid and accurate method to identify haploid maize kernel using near-infrared hyperspectral imaging technology to overcome the limitations of current automated haploid identification and to achieve more accurate screening of haploid. In terms of two representative varieties of maize (Zhengdan 958 and Nongda 616), the study adopted spectral features of hyperspectral imaging to discuss the influence of embryonic orientation (embryo faces to or against light source) on haploid identification model. Meanwhile, the separability of embryo and non-embryo and identification accuracy of joint modeling of embryo and non-embryo were analyzed. The study showed that the greater difference between embryo and non-embryo of haploid and diploid, but hyperspectral imaging method could effectively distinguish haploid and diploid through embryo or non-embryo. At the same time, with the qualitative analysis method, two maize varieties could accurately distinguished haploid and diploid with overlapping oil content based on joint modeling. In this case, the test set of haploid and diploid achieved yielded higher correct acceptance rate (CAR) of 99% and the false acceptance rate (FAR) were both below 1%, with a high accuracy rate. The study showed that it is feasible to recognize maize haploid using hyperspectral imaging technology, which can provide a reference for the later haploid sorting systems.

1. Introduction

Haploid breeding in maize breeding has very broad applications (Ma et al., 2011). Haploid breeding approach combining biotechnology with conventional methods, effectively accelerate the process of maize breeding, thereby promoting greater efficiency of maize breeding while saving the cost of maize breeding, and also have important significance for germplasm improvement (Dwivedi et al., 2015; Sun et al., 2009). Since the probability that maize produces haploid in natural conditions is low, generally no more than 0.1%, even if the haploid produced by artificially induced is also less than 10% (Li et al., 2016a; Qin et al., 2016). That is to say, in practice, the number of diploid is more than that of haploid, the ratio is about 9:1. In order to achieve haploid maize breeding, it is necessary to select the haploid kernels from a large number of mixed kernels of haploid and diploid, so it is very crucial to rapidly and accurately recognize haploid maize kernels from a large

Abbreviations: NMR, nuclear magnetic resonance; CAR, correct acceptance rate; FAR, false acceptance rate; ROIs, region of interests; BULDP, biomimetic uncorrelated locality discriminant projection; BPR, biomimetic pattern recognition; PCA, principal component analysis

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number of mixed kernels for haploid breeding.

In haploid maize kernel identification field, two conventional and non-destructive haploid identification technologies have been developed, respectively genetic marker method based on *R1-nj* pigment gene (Nanda And Chase, 1966) and oil content marker method based on oil xenia effect (Chen and Song, 2003). Genetic marker method uses pigment gene to express pigment in induced hybrid kernels, and the embryo of haploid has no color marker, the embryo of diploid has a color marker, and therefore, human eyes can directly and easily select haploid. However, the selection of haploid by human eyes is highly based on subjective experience. Meanwhile human eyes are apt to be fatigue, and kernels with unclearly expressed color of embryo and endosperm aleurone layer are hard to be recognized (Li et al., 2016a; Qin et al., 2016). Thus, such selection method consuming excessive time and efforts is unable to meet breeding demand. Therefore, the haploid maize kernel identification technology where machine vision is used to recognize genetic marker of color was presented (De La Fuente et al., 2017; Li et al., 2016b; Zhang et al., 2013). Such technology recognizes embryonic color marker by acquiring embryonic images of maize kernels. However, such technology applies to the circumstance that the embryo of maize kernel is upward only. And kernels with unclear color marker expression are challenging for both machine vision and selection with human eyes. This limits generalization of haploid classification and selection technology to some degree. Oil content marker method makes haploid and hybrid diploid kernels prominently different in oil content through hybrid between high oil inducer and non-high oil material. At present, nuclear magnetic resonance (NMR) is used to recognize haploid by the oil content of kernel (Liu et al., 2012; Melchinger et al., 2014; Wang et al., 2016). However, the oil content of haploid and diploid is overlapped, in order to exclude diploid as far as possible, the choice of oil content threshold will sacrifice the number of haploid to a certain extent and cause the waste of haploid kernels (Li et al., 2016a). Therefore, the problems encountered by the current methods limit the application of haploid automated identification technology.

Hyperspectral imaging technology is a new non-destructive inspection method which combines imaging technology and spectral analysis technology. Hyperspectral image consists of images at a series of wavelengths. There is a specific two-dimensional image at each wavelength. Additionally, the grey information of the same pixel point at different wavelengths also provides spectral information (Zhao et al., 2011). Compared with conventional machine vision and NMR, hyperspectral imaging technology can obtain color, morphology, texture and other external features of samples, and information about chemical elements in kernels to analyze kernels in numerous aspects (Tang et al., 2015). Williams et al. combined hyperspectral imaging with multivariate data analysis to achieve early fungal contamination detection of corn seeds (Williams et al., 2012). Huang et al. used hyperspectral imaging to analyze maize kernels of different years, and found by means of model updating could achieved the classification accuracy of 94.4% for maize kernels of different years (Huang et al., 2016). Serranti et al. studied the rapid classification method of three types of wheat (yellow berry, vitreous and fusarium damaged) using hyperspectral imaging techniques, and obtained good classification performance (Serranti et al., 2013). To overcome the existing problems of automatic identification of haploid, thus, in this study, near-infrared hyperspectral imaging technology is used to explore the feasibility of haploid maize kernel identification.

To rapidly and correctly recognize haploid maize kernels using hyperspectral imaging technology, two aspects should be considered:

(1) To fulfill rapid haploid maize identification and analyze without human involved, impacts of different parts of maize kernel (embryo and non-embryo) on identification performance of the system was studied, and explore the method to accurately identify the different poses and placements of kernels, and identification model was established to test and evaluate performances of the system.
(2) The situation that haploid and diploid have overlap of oil content will exist when oil markers were used. The quantitative analysis method with oil as an indicator, and threshold value selection method would cause low accuracy, so it's necessary to explore the qualitative analysis method to extract the effective information sufficiently and realize the classification accuracy of haploid and diploid with overlapping oil content. The identification model should ensure that haploid can be identified as much as possible and have enough ability to reject diploid in the mixed kernels.

2. Materials and methods

2.1. Experimental samples

The experiment utilized two representative samples, namely, Zhengdan 958 and Nongda 616, respectively. Each variety includes haploid and diploid kernels. The experimental samples were produced from high oil hybrid induction with *R1-nj* genetic marker was induced and supplied by National Maize Improvement Center of China Agricultural University. One hundred kernels of haploid and one hundred kernels of diploid were selected from Zhengdan 958 as research object, one hundred kernels of haploid and one hundred kernels of diploid were selected from Nongda 616 as verification object. Each kernel was numbered in the experiment. The difference of genetic marker between haploid and diploid of Zhengdan 958 and Nongda 616 is shown in Fig. 1. It can be seen that there are no color genetic markers on the haploid embryo of the two maize varieties, and color genetic

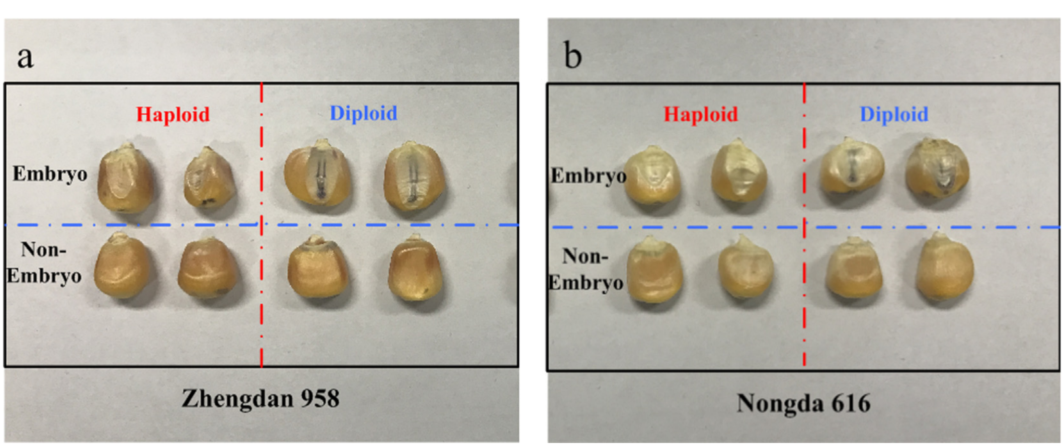

Fig. 1. The genetic markers of haploid and diploid: (a). Zhengdan 958; (b). Nongda 616.