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A high-throughput maize kernel traits scorer based on line-scan imaging

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ABSTRACT

Maize kernel traits such as kernel length, kernel width and kernel number determine kernel weight and, consequently, maize yield. Therefore, the measurement of kernel traits is important for maize breeding and the evaluation of maize yield. The conventional method for measuring kernel traits is still manual, which is time consuming, costly and subjective. In this study, a novel maize kernel traits scorer (MKTS) was developed for the automatic measurement of 12 maize kernel traits based on line-scan imaging, image processing, and automatic control techniques. Here, total of 615 samples were measured to evaluate the system performance. The results showed that the MKTS was capable of evaluating maize kernel traits with the mean absolute percentage error of the manual and automatic measurements less than 5% and the measurement efficiency of approximately 72 s for the measurement of 6 ears. In conclusion, this high-throughput scorer will provide maize scientists with a novel tool to assist in maize functional genetics and maize breeding.

1. Introduction

Maize (*Zea mays* L.) is a primary food, feed and fuel worldwide [1,2]. To meet the demands of the world's growing population, improvement in maize yield and quality through the combination of traditional and molecular breeding is urgently needed [2]. Kernel traits are important in maize breeding programs. Such traits include kernel yield [3], which is determined by the ear number per plant, the kernel number per ear and the kernel weight [4–6]. The kernel weight is one of the most important agronomic traits; it is determined by kernel shape, including the kernel length, kernel width, and kernel thickness [7]. With the development of next generation sequencing technologies, the generation of high quality genotype data has become extremely feasible in maize. To dissect the genetic basis of maize kernel traits, the accurate measurement of maize kernel traits is crucial and advantageous in functional genomics research and genetic improvement in maize.

The conventional method for measuring and recording maize kernel traits is still manual, which is time consuming, costly, and subjective [8,9]. Plant phenomics is a multidisciplinary field that combining of mechanics, automatic control, photonics-based techniques, and digital image processing to plant science study [10]. Digital imaging has been widely used in agriculture, such as in the detection of rice yield-related traits [7,11], the determination of the surface color of agricultural products [12–14], the detection mechanical damage in kernels [12,15,16], locating insects in cereal grains [17], mapping of seed shape/size QTL [4–6,18], and the evaluation of grain quality [19–23]. For maize, phenomics is used to evaluate the quality of kernels, classify maize kernels into size categories, and measure kernel shape. Valiente-Gonzalez [24] designed a computer vision system to capture single dent corn kernels and determine whether kernels were damaged using a principal component analysis (PCA) algorithm. Xun et al. [25] developed an on-line seed grading system based on machine vision; corn seeds were sorted into four grades according to morphological parameters, with an average eligible grading ratio of 81.90%. Steenhoek and Precetti [26] evaluated the use of two-dimensional image analysis for the classification of maize kernels according to size with accuracy greater than 96% for round-hole decisions and less

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Abbreviations: MKTS, maize kernel traits scorer; EN, ear number; KL, kernel length; KW, kernel width; LWR, kernel length-width ratio; KPA, kernel projected area; KPP, kernel projected perimeter; TKN, total kernel number; TKW, total kernel weight; 100-KW, weight per 100 kernels; MAPE, mean absolute percentage error; KWPE, kernel weight per ear; KNPE, kernel number per ear; MVPA, mean value of the projected area; $STDEV_{APE}$, standard deviation of APE; C.V, coefficient variation; RMSE, root mean squared error; APE, absolute percentage error; MR, misjudgment rate.

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than 80% for flatness decisions. Ni et al. [27] developed an electronic corn kernel size grading system based on machine vision and measured kernel length, width and projected area. However, the kernels were individually measured in stationary way, and the processing time was between 2.03 and 2.09 s per kernel. Severini et al. [28] developed a method for counting maize kernels using the open source software ImageJ, but maize kernels were spread over the sample platform by hand. Recently, with manual spreading samples, some researchers measure particle shape/size using image processing software such as ImageJ and SmartGrain. Igathinathane et al. [29] developed an ImageJ plugin to identify disjoint particles shape and determine their particles size distribution. Tanabata et al. [30] developed SmartGrain software for high-throughput measurement of seed shape. To the best of our knowledge, little effort has been undertaken for the high-throughput extraction of maize kernel traits in commercialized products.

In this study, automatic control and image analysis techniques based on line-scan imaging were used to develop a high-throughput maize kernel traits scorer (MKTS) to measure maize kernel traits. These traits included ear number (EN), kernel length (KL), kernel width (KW), the length-width ratio (LWR), kernel projected area (KPA), kernel projected perimeter (KPP), the total kernel number (TKN), the total kernel weight (TKW), the weight per 100 kernels (100-KW), the kernel weight per ear (KWPE), the kernel number per ear (KNPE), and roundness. Equipped with image processing and automatic control technologies, this novel instrument provided a high-throughput and high-accuracy method for maize kernel scoring, and has potential to be popularized in maize functional genetics, genomics and breeding.

2. Materials and methods

2.1. Materials

In this study, a total of 615 different maize samples were used in this study. From these 615 samples, 20 maize kernels with uniform areas were chosen to evaluate the system performance for measuring the kernel shape (KL, KW, and LWR) and the other remaining kernels were used to evaluate the system performance for measuring the TKN, TKW, and 100-KW. For the manual measurement of the KL and KW, the length and width of each kernel of 20 maize kernels with uniform areas were measured with three people using a digital vernier caliper, and the average value was calculated. For the manual measurement of TKN, each sample was counted with three people and the average was calculated. After measured manually, all the 615 maize samples were measured automatically using the MKTS system. Moreover, 10 different maize samples were chosen randomly from the 615 samples to validate the repeatability of the MKTS. The experimental design was show in the Supplementary file 1.

2.2. System description

The user operation area and a prototype of the system are shown in Fig. 1a. The operation area included a software interface, a barcode scanner, a feeding interface and a vibrating feeder. When maize kernels were manually placed in the feeding interface, the samples were delivered to the inspection unit (Fig. 1b) by the vibrating feeder. The inspection unit was designed with the following three key components: a line-scan camera with short-focus lens, a line-array LED light source and a conveyor with a servo motor. To acquire maize kernel images continuously and shorten image processing time, a line scan camera was applied instead of a conventional frame camera because merging kernel images is easier with a line scan camera than that of frame camera. To achieve high-throughput and dynamic measurements, an industrial conveyor driven by the servo motor, which was blackened to enhance contrast between the maize kernels and the background, delivered maize kernels through the imaging area automatically. The two line-array LED light sources provided uniform illumination. The inner layout of the system is illustrated in Fig. 1c; this included the inspection unit, a power adapter for the light source, a programmable logic controller (PLC), a computer, a feeder driver, an electronic scale and a collection box. The feeder control, the conveyor control and communication with computer were achieved via the PLC. The collection box was used to collect the measured maize kernels. The TKW was obtained via the electronic scale. The details of the component used in the maize kernel traits scorer (MKTS) were shown in the Supplementary file 2. The operation procedure video of the MKTS was shown in the Supplementary file 3.

2.3. Operation procedure and system controls

The system operation procedure (shown in Fig. 2a) included the following steps: (1) Start the system and scan the barcode; (2) input the ear number; (3) put the kernels into the feeding interface and start the inspection; (4) allow each frame image to be acquired and delivered to the queue for image processing; (5) after all the kernels were scanned, end the current task and acquire the total kernel weight with the electronic scale; and (6) find the results, including the original gray image and the maize kernel traits, stored in the user-predefined folder. The PLC was programmed using CX-Programmer 7.3 (Omron, Japan), the software for the computer was developed using LabVIEW 8.6 (National Instruments, USA), and the software for statistics analysis of maize kernel traits was SPSS (version 19.0, International Business Machines Corporation, USA).

2.4. Image processing and the extraction of traits

A flow chart of the image processing used for the measurement of maize kernel traits is outlined in Fig. 2b. After each kernel was captured by the line-array camera, the kernel images were sent to a queue. Image processing was synchronous with image acquisition, which included the following steps: (1) N frame images were fetched from the queue and a non-adaptive thresholding algorithm (the threshold value was 10) was applied to divide the gray image into a background and foreground for each image frame; then several morphological operators (including open and filling holes) were used to process the binary image; (2) the processed image was split into two parts, including a cut part and a remaining part; (3) the remaining part of the N image was merged with the cut part of the former image to obtain a merged image; (4) the mean value of the projected area (MVPA) of all objects was obtained and the objects with a projected area less than 0.4 times the MVPA were not considered as maize kernels and removed using particle filter operator; (5) then the Elongation Factor for all kernel samples (1.4–3.4), which was defined as maximum diameter divided by equivalent rectangle short side, was calculated and the objects with Elongation Factor more than 4 were not considered as maize kernels and removed using particle filter operator; (6) the projected area of kernels was used to classify touching and non-touching kernels, which the objects with a projected area more than 1.45 times the MVPA were considered to be touching objects, and the remaining objects were considered to be non-touching objects; (7) with those non-touching kernels, all pixels of all non-touching kernels were summed and divided by the number of those non-touching kernels, then multiplied by the spatial resolution (0.02295684 mm^2/pixel) would be the MVPA; all pixels of all non-touching kernels' edges were summed and divided