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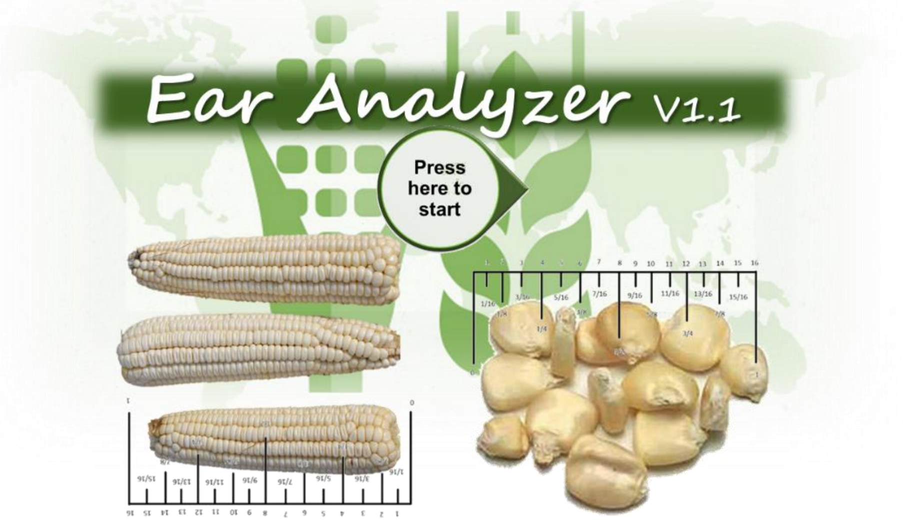
International Maize and Wheat Improvement Center



MAIZE EAR PHENOTYPING USING DIGITAL EAR IMAGING

Global Maize Program

CIMMYT

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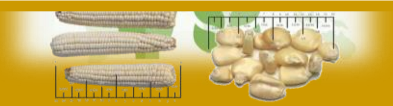
User Guide

CIMMYT-Harare, Zimbabwe

CIMMYT



Acknowledgements

The information presented in this manual is based on the phenotyping and breeding work that received strong and consistent support from several donor agencies, especially the USAID, BMGF, DFID, GIZ, and the MAIZE CGIAR Research Program, as well as public and private sector partners in sub-Saharan Africa.

CIMMYT – the International Maize and Wheat Improvement Center is the global leader in publicly-funded maize and wheat research-for development. Headquartered near Mexico City, CIMMYT works with hundreds of partners worldwide to sustainably increase the productivity of maize and wheat cropping systems, thus improving global food security and reducing poverty. CIMMYT is a member of the CGIAR Consortium and leads the CGIAR Research Programs on MAIZE and WHEAT. The Center receives support from national governments, foundations, development banks and other public and private agencies.

Contents

Preface

Grain yield, ear and kernel attributes can assist to understand the performance of maize plant under different environmental conditions and can be used in the variety development process to address farmer's preferences. These parameters are however still laborious and expensive to measure.

The ear digital imaging is a low-cost, high throughput method that provides estimates of ear and kernel attributes i.e., ear number and size, kernel number and size as well as kernel weight from photos of ears harvested from field trial plots. The image processing method uses a script that runs in a batch mode on ImageJ; an open source software.

Current investment in combine harvesters at key breeding locations may supersede this technology; however it will continue to provide an important quality control feedback in on-farm trials where yields are generally measured by non-researchers. Furthermore, the cost and maintenance of harvest equipment does not make them accessible for small breeding programs, especially within national programs.

This manual is designed to assist maize breeders and technicians in ear and kernel attributes evaluation.

It covers aspects related to:

- *ImageJ software installation,*
- *Image acquisition procedure,*
- *Image processing steps.*

Background

In maize, yield is a function of interdependent characteristics of ears and kernels [1]. A well-developed maize ear may have close to a thousand kernels [2]. The number of kernels per ear is a function of ear width (kernels per row) and kernel rows per ear. Many stresses can affect row number and kernels per row, as well as kernel size/weight. Cairns et al. [3] reported that under drought conditions, yield loss in both hybrids and inbreds was largely associated with a highly significant decrease in the number of kernels per unit of ear area. Plant water deficit at flowering has been shown to negatively affect kernel number [4] and deficiencies in N supply usually decrease grain yield by lowering kernel number per plant [5, 6] as a result of less synchronous pollination [7], and/or greater kernel abortion [8]. This indicates that these ear and kernel features can be used to assess the tolerance of a variety to a stressful condition. From a breeding perspective, studies have found that yield components tend to display greater heritability than overall yield [9, 10]; making it possible to select for these traits separately and then combine the responsible genetic loci to develop a genotype with superior performance or develop a selection index through traits combinations [11].

There are few methods that allow the extraction of ear and kernel features through image processing. A method of evaluating one or more kernels of an ear of maize using digital imagery was patented by Pioneer (Hi-Bred International, inc., Iowa) in 2009 [12]. The method enables to extract kernel count, kernel size distribution, proportion of kernels aborted and other information using image processing algorithms that include, without limitation, filtering, watershedding, thresholding, edge finding, edge enhancement, color selection and spectral filtering. Zhao et al. [13] have proposed a method that provides kernel counts from ear photos, with the assumption that a maize ear has double the number of rows and kernels than can be visible on a photo. More recently, Liang et al. [14], have developed a method that scores maize kernel traits under controlled lighting conditions. based on line-scan imaging. In addition, Miller et al. [1] have proposed three custom algorithms designed to compute kernel features automatically from digital images acquired by a low cost platform.

The main limitation of these methods is that they often rely on systems like a scanner that have controlled lighting conditions and fixed image background. In addition, they do not provide a comprehensive data set from a single image of unthreshed ears (i.e. ear count, ear and kernel features) taken under field conditions simultaneously and in an automated manner.
Although there are harvesting equipments that automatically measure grain yield on a plot level, yield component traits such as ear and kernel dimensions are usually measured by hand [15, 16, 17]. In addition, this kind of equipment is quite expensive to buy and maintain, therefore not affordable for most breeding programs, especially in sub-Saharan Africa.

Image acquisition

Ears should be harvested, de-husked and kept per plot. They should be arranged on a black piece of cloth side by side in a way that they are not in much contact with each other. In the case that the soil where the trial was conducted has enough contrast with the colour of the ears, they can be arranged directly on the soil (Fig. 1a). Images of all ears belonging to a plot should be taken using a consumer grade digital camera set in automatic mode. The images can also be captured using a mobile phone or tablet provided that their camera have a resolution of at least 8 megapixels. The camera can be used directly or mounted on a tripod stand and positioned at nadir (Fig.1). The tripod option is recommended because it enables the image processing to be done in a batch mode (*when using the tripod option, the use of a Wi-Fi enabled camera would make the image acquisition process easier as the camera can be remotely controlled from a mobile phone or tablet*). **It is very important that an image of a ruler or any object with known size be taken at the same height as the ear images, to convert the pixel scale measurements to centimetres.**

Figure 1: Image acquisition procedure using (a) bare soil and (b) black piece of cloth as background.

Image processing

The image analysis procedure is conducted in imageJ (https://imagej.nih.gov/ij/features.html), an open source software using a script (***Ear Analyser***) developed at CIMMYT. The key steps for performing the image processing are described below:

1. Installing the ImageJ software

1.1. **Download the ImageJ** (also called FIJI) software version that is compatible with your operating system from the link below:

https://imagej.nih.gov/ij/features.html

The software will be save in a ZIP archive format. Unzip the ImageJ folder in your directory of choice (it could be for example in *Documents*).

1.2. Place the ***Ear Analyser*** file in the “Analyze” folder located in ImageJ plugins folder:

C:\Users\Documents**ImageJ\plugins\Analyze**

2. Processing the images

2.1. Open the ImageJ software using ***ImageJ.exe*** command located in ImageJ folder (Fig. 2).

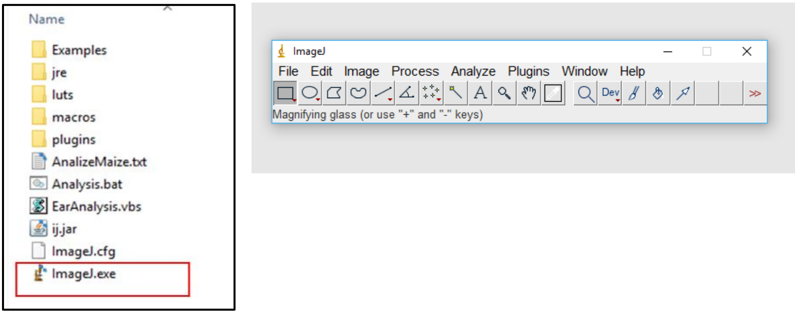


Figure 2: Opening the ImageJ software procedure.

2.2. Setting the scale

To set the scale, open the image of a ruler (or any object with known size) taken at the same height as the ear images. Use the *freehand lines* tool from the toolbar and draw a line measuring 1 cm on the ruler as shown in Fig. 3. Note down the equivalent value in pixels (in this example length= 47.50). Then close that image.

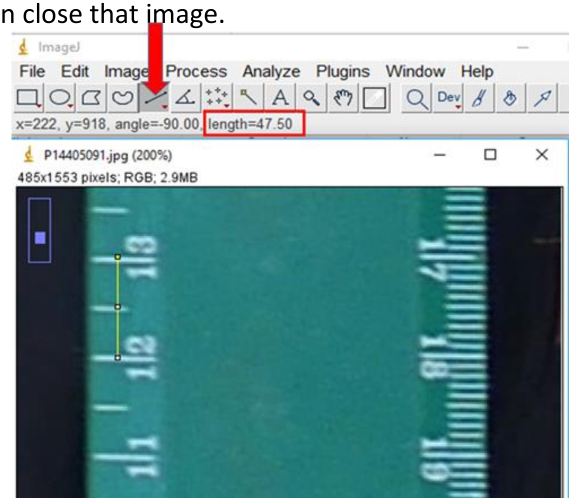


Figure 3: Procedure for setting the scale.

2.3. Running the plugin

To run the ***Ear Analyser*** plugin, from the ImageJ menu, go to *Plugins*, then *Analyse* and select *Ear Analyser* (Fig. 4).

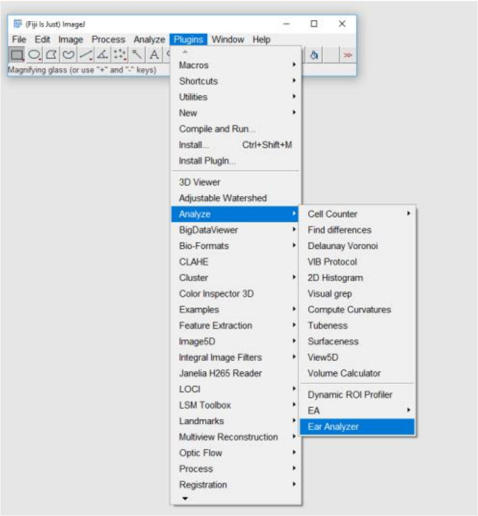


Figure 4: Procedure for running the *Ear Analyser* plugin.

A small window will be open up requesting to input the distance in pixels for 1 cm (Fig. 5). Put the value that you have noted in the setting the scale section.

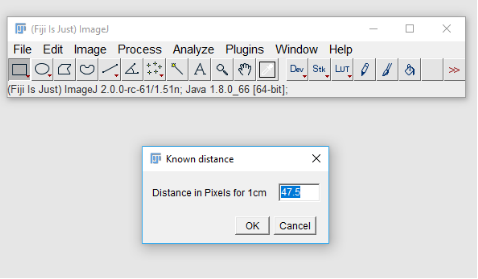


Figure 5: Procedure for scale setting.

In the next step, you will be asked to indicate the location of the folder that contains your images (Fig. 6). It is recommended to have all the images taken at the same height in the same folder. This will enable to process all the images at once.

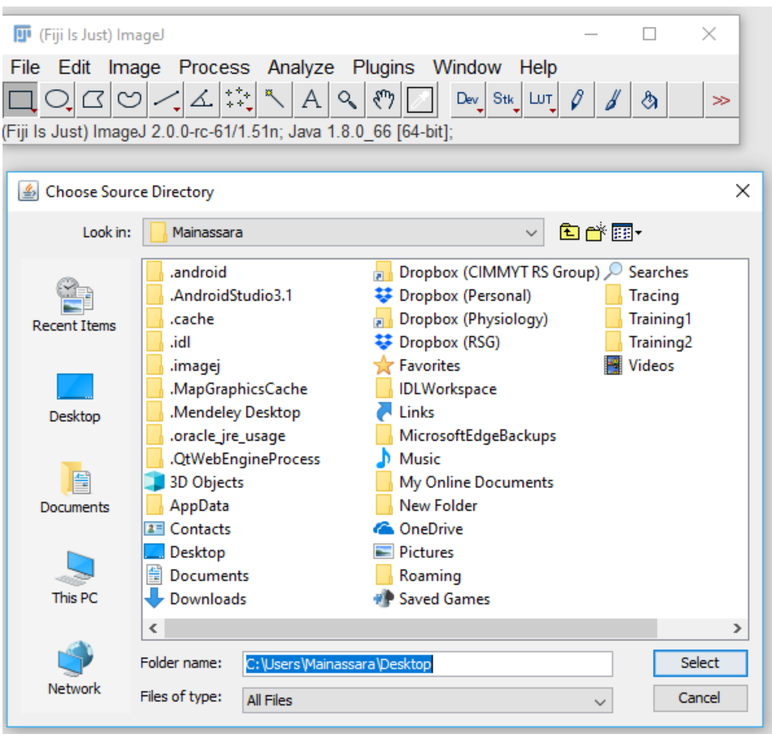


Figure 6: Procedure for source directory choice.

After selecting the folder, the processing will automatically begin and continue until the last image in the folder is processed. A result table will appear capturing the data output progressively (Fig. 7). The data extracted from the images will be sorted based on original image caption and saved as CSV file that can be opened with excel. Once the processing is finished, make sure to save your output data file using a name that relates to your trial name to be able to easily find it when searching.

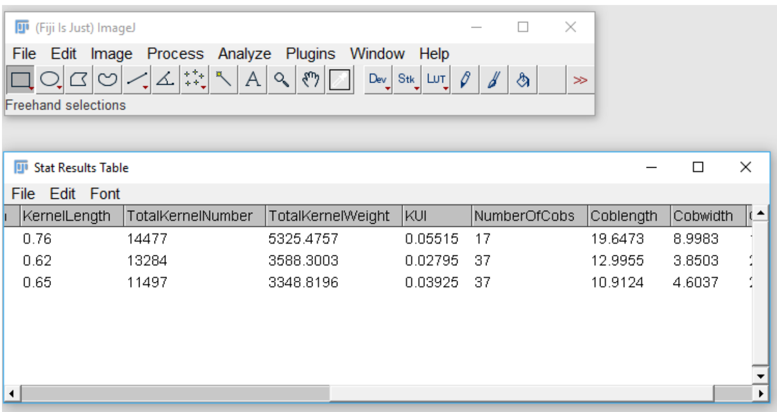


Figure 7: Result table showing output data.

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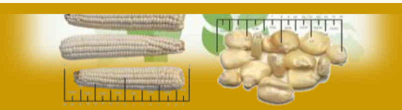

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