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Evaluation and comparison of open source program solutions for automatic seed counting on digital images

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ABSTRACT

Seed number quantification is an essential agronomic parameter conducted mostly manually or by mechanical counters, both with obvious limitations. Digital image analysis provides a reliable and robust alternative to accurately calculate many biological features. This study presents and evaluates the performance of four open-source image-analysis programs i.e. ImageJ, CellProfiler, P-TRAP and SmartGrain to count crop seeds from digital images captured by camera and scanner. It also evaluates ImageJ program for automated seed counting using macro containing RenyiEntropy threshold algorithm. Digital images of cereal crop seeds were acquired i.e. wheat, barley, maize, rye, oat, sorghum, triticale and rice. All images contained 200 seeds per image present in an area of approx. 1400 cm^2. RenyiEntropy threshold increased the seed count accuracy of ImageJ from digital camera images. Generally, seed counts from digital camera images of all crops were accurate, but software–crop combination had significant ($p < 0.05$) difference from reference value. Among image analysis programs, ImageJ produced mostly higher seed count across all observed crops than other programs. Mean seed counts from scanned images of maize were observed only by CellProfiler and P-TRAP, with other programs inappropriate due to high inaccuracy. These results suggest CellProfiler as a reliable image analysis program for seed counting from digital images. Benchmark test was also performed to compare speed of analysis. The automated seed count produced by image analysis programs described here allows faster, reliable and reproducible analysis, compared to standard manual method. To our knowledge this is the first study on using CellProfiler program for crop seed counting from digital images.

1. Introduction

Researchers devote copious efforts to make use of available technology for reliable estimation of desired experimental parameters in a fast, inexpensive and efficient way. In the past few years, we have seen an emerging use of digital imaging technology for various sensitive measurements. There has been an extensive use of image analysis programs to perform measurements that look laborious and expensive in the past. One of these processes is target object or particle counting from digital images. This capability has been applied in various fields to count for example, neurons (Oberlaender et al., 2009), pollens (Costa and Yang, 2009), root structures (Tajima and Kato, 2011), and fruits on trees (Payne et al., 2014). In addition to this, image analysis has an ever-increasing use in various scientific fields for example to estimate plant nitrogen concentrations from digital images (Rorie et al., 2011) for efficient nitrogen management.

The approximation of seed count is a common agronomic parameter. Seed number is an important determinant of grain yield and also monitored during postharvest processing (Bilsborrow et al., 2013). Numerous methods have been developed to count number of seeds from given samples. These studies date back to development of photoelectric seed detector (Reid et al., 1976) and evolved to a completely automated method described by Severini et al. (2011). Seed counting is mostly performed manually using naked eye which can be very time-consuming and involves high chances of human error or by use of electronic or laser-based particle counters, which can be expensive as well.

An open-source Java-based ImageJ program (Schneider et al., 2012) has been used in a wide range of studies relating to image analysis e.g. counting neuron contacts (Wouterlood et al., 2008), shape and particle size distributions (Igathinathane et al., 2008) and bone geometrical measurements (Doube et al., 2010).

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Specific extensions, different plugins and macros are available to download from United States National Institute of Health webpage for ImageJ. ImageJ has proved the ability to automatically count crop pollens (Costa and Yang, 2009) or seeds (Severini et al., 2011) by running a macro. However studies are lacking on the program performance from scanned images.

Another open-source (GPL, General Public License) program, CellProfiler (Carpenter et al., 2006) reported in scientific studies e.g. stained comets of DNA (González et al., 2012) and morphometric analysis of leukocytes (Tozetti et al., 2014). CellProfiler features a user customizable pipeline of modules that contain a variety of algorithms to automatically identify, quantify and export the assay information. These modular pipelines can be saved for subsequent runs.

P-TRAP (Panicle Trait Phenotyping) is recently developed open-source Java based program (AL-Tam et al., 2013) purposed for automatic measurements of rice panicle architecture and seed-related traits. It has also been used to study variation in rice inflorescence development (Crowell et al., 2014).

SmartGrain is an open-source program recently developed for high-throughput phenotyping of rice seeds by the National Institute of Agrobiological Sciences, Japan (Tanabata et al., 2012). It has been written in Visual C++ in the Microsoft Visual Studio 2010 and runs only under Microsoft Windows platforms. It has been used to measure grain size and shape to identify genes controlling those features (Okamoto et al., 2013).

The aim of the study was to evaluate four open-source image analysis programs: ImageJ, CellProfiler, P-TRAP and SmartGrain for ability to identify and count the seed numbers from digital images. Another objective of the presented work was to validate RenyiEntropy threshold algorithm in ImageJ for seed counting. It was also evaluated whether automated procedures can effectively contribute to crop analysis by decreasing workload and inspection time.

2. Materials and methods

2.1. Plant material

Eight different cereal crops (Table 1) i.e. wheat, maize, barley, rice, rye, oat, triticale and sorghum were used in the study. The seed samples of selected crops were manually prepared by removing any dirt or inert material and subsequently subjected to digital imaging.

2.2. Image acquisition

To explore performance of selected open-source image analysis programs for seed counting, digital camera images ($n = 30$) were acquired for all selected crops. Sets of 200 seeds per image were spread within an area of 1400 cm^2 in a way that the particles neither overlap nor touch one another. For a better contrast, a black background was used while acquiring the images. A Canon Powershot A3100 IS (Tokyo, Japan) was adjusted to f/2.7, 1/60 s, ISO 80, 6.2 mm resulting in 180 dpi images of JPEG format images as shown in examples image (Fig. 1). Camera flash was turned ON, but was covered all the time with white opaque layers of commonly available polyethylene plastic from food bags, to provide diffuse illumination.

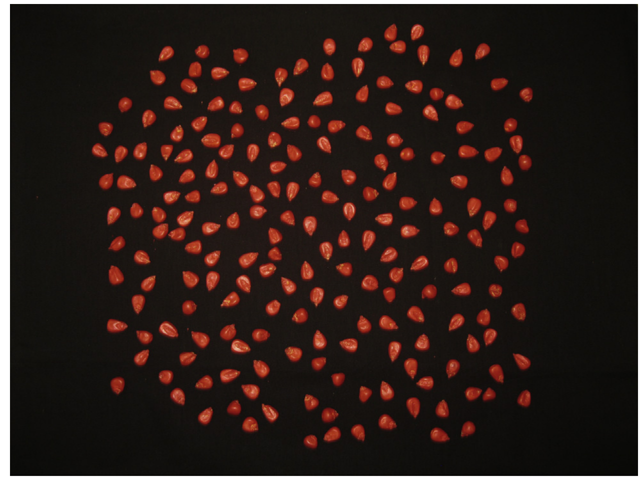

Fig. 1. A sample image of maize containing 200 seeds spread within 1400 cm^2 used for seed counting by four open-source digital image analysis programs.

Scanned images of maize ($n = 30$) were also acquired using a HP Deskjet F2180 flatbed scanner (Hewlett–Packard, USA) with a black background resulting images of 200 dpi. This second image set was used to test influence of different acquisition methods on accuracy.

2.2.1. Image processing in ImageJ

Seed counting from digital images using ImageJ (v 1.48) program was automated by running a macro first reported in Severini et al. (2011). However, in the present study the threshold of Binary function used in Severini et al. (2011) was replaced to a more sophisticated "RenyiEntropy threshold" (Sahoo and Arora, 2004) aimed to reduce image noise more effectively. Images were to be automatically processed (i.e. convert to 8-bit gray scale image, auto threshold, watershed and analyze particle) by modified macro (Fig. 2).

ImageJ requires a user input of minimum particle size (pixel) to estimate the correct seed number from digital images. The minimum threshold values of particle size for crop seeds were selected by processing 30 digital camera images in ImageJ. This was done by running the macro (RenyiEntropy threshold) across a range of minimum particle size (25–115 pixel) and identifying the range with minimum percentile difference from the reference count as shown for example for barley in Fig. 3.

Table 1
Crops used in the study.

No.	Crop name	Botanical name	Variety	Source
1	Maize	*Zea mays* L.	Supra	Saaten Union Intl.
2	Wheat	*Triticum aestivum* L.	Mv Kikelet	Mv Elitmag Ltd. Hungary
3	Rye	*Secale cereale* L.	Varda	Vetőmag
4	Barley	*Hordeum vulgare* L.	KH Tas	Agromag, Hungary
5	Sorghum	*Sorghum bicolor* L.	ES Queyras	Euralis, Hungary
6	Oat	*Avena sativa* L.	Mv hopehely	Mv Elitmag Ltd. Hungary
7	Rice	*Oryza sativa* L.	Basmati	SZIE, Crop Science Lab.
8	Triticale	*Triticosecale*	Tatra	Agrozen