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Research paper

Image-based rapid phenotyping of chickpeas seed size

Sindhuja Sankaran [a,*], Meng Wang [a], George J. Vandemark [b]

[a] Department of Biological Systems Engineering, Washington State University, Pullman, WA 99164, USA
[b] Grain Legume Genetics and Physiology Research Unit, Pullman, WA 99164, USA

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ABSTRACT

In chickpeas, the seed size is a critical phenotype that needs to be evaluated carefully during variety development. The sieve analysis used for determining seed size distribution in legumes is labor intensive and time consuming method. An image-based method for sizing chickpeas seeds was developed in this study. Samples from a total of 72 plots from two different locations were harvested and seed size was analyzed. The results show that seed size calculated from image-based method was highly correlated to the ground-truth data, with a correlation coefficient of 0.90. The image processing technique provides rapid evaluation of seed size for phenotyping chickpeas and the method also can be adapted for similar seed types.

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1. Introduction

Chickpea (*Cicer arientinum* L.) is considered one of the "founder crops" domesticated by Neolithic cultures in the Near East 12,000–8,000 years ago (Hopf and Zohari, 2001). Chickpeas are typically grown in rotation with small cereal grains such as wheat and barley. This benefits cereal production due to the disruption of cereal disease cycles and making available to cereal crops residual nitrogen produced through biological nitrogen fixation by rhizobacteria that reside in chickpea roots. The complementation between chickpeas and cereal grains extends to nutritional qualities. Chickpea has relatively high seed concentrations of lysine and low concentrations of the sulfur-containing amino acids methionine and cysteine, whereas cereal grains tend to have low concentrations of lysine and high concentrations of methionine and cysteine (Bressani and Elias, 1988). Chickpeas can be divided into two major classes, the macrosperma, or 'kabuli' class, and the microsperma, or 'desi' class (Toker, 2009). Kabuli chickpea seeds are considered to have the shape of an 'owl head' and are larger and lighter in color than desi type chickpeas, which have a 'teardrop' seed shape. Kabuli chickpeas are cooked and used for salads, canned and eaten whole, or used to make hummus, while desi chickpeas are predominately split and then cooked in soups and stews (Newman et al., 1988). In the USA, commercial production is almost exclusively limited to kabuli chickpeas (Vandemark et al., 2014).

Chickpeas are grown in the USA primarily in WA and ID, with lesser amounts in CO, MT, NE, ND, and CA. Over a recent three year period (2012–2014), an average of over 50×10^7 kg of chickpea was harvested annually in the USA across more than 833 square km (NASS, 2015). The value of a chickpea crop is influenced by both total seed yield and also by the size of the harvested seed (Upadhayaya et al., 2006). Larger seeds are used for canning, salads, and fresh markets and have a higher value than smaller seeds, which are typically processed into hummus. The USDA National Agricultural Statistics Service (NASS, 2015) separates chickpeas into two size classes based on the diameter of seed: small (<7.9 mm), and large (≥7.9 mm). Over a recent three year period (2012–2014), large chickpeas have received an average price of \$0.85 kg^{-1}, which is 51% higher than the average price of \$0.56 kg^{-1} received for small chickpeas (NASS, 2015). In addition to seed size, the uniformity of seed size among a sample of harvested chickpeas can impact both agronomic considerations, such as accuracy of seeding rates, along with post-harvest traits such as water absorption of seeds, which is of special importance to the canning markets (Shahin and Symons, 2005; Firatligil-Durmus et al., 2010). Therefore, seed size and distribution of seed size are critical traits that need to be carefully evaluated during the process of chickpea population improvement in order to develop new varieties that have desirable commercial traits.

The sieve analysis is a standard method for determining seed size distribution in grains (FGIS, 2014). In general, desired screen sizes are selected to categorize the seeds having specific sizes, and the percent size distribution is determined by weight. Both manual

* Corresponding author.
E-mail address: sindhuja.sankaran@wsu.edu (S. Sankaran).

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and mechanical sieving are laborious and time-consuming, and can sometimes damage the seeds. In this regard, the image-based seed sizing could be a rapid, efficient and non-invasive technique for evaluating seed size. Although, such techniques have discussed by researchers (Shahin and Symons, 2005; Herridge et al., 2011; Fıratlıgil-Durmuş et al., 2010; Kumar et al., 2013), no assessment or development of the imaging techniques with large data sample size exists. Moreover, it is also important to compare the image seed size with ground-truth data used by plant breeders for decision-making. Therefore, the overall goal of this work was to evaluate the image-based seed sizing technique for phenotyping chickpeas seeds, and develop an algorithm that can be used or adapted by other researchers for similar seed analysis.

2. Material and methods

2.1. Field plots

Yield trials were conducted in 2013 that included 20 advanced kabuli chickpea breeding lines from the USDA-ARS chickpea breeding program in Pullman, WA and four commercial check cultivars. The check cultivars included three kabuli types ('Sawyer', 'Sierra', and 'CDC-Frontier') and a small chickpea landrace ('Billybean'). Trials were planted at two locations: Dayton, WA on May 6, 2013, and Pullman, WA on April 29, 2013. Prior to planting, all seeds received a seed coat treatment applied at a rate of 30 mL kg^{-1} seed that contained the fungicides fludioxonil (0.56 g kg^{-1}, Syngenta, Greensboro, NC), mefenoxam (0.38 g kg^{-1}, Syngenta, Greensboro, NC), and thiabendazole (1.87 g kg^{-1}, Syngenta, Greensboro, NC), thiamethoxam (0.66 mL kg^{-1}, Syngenta, Greensboro, NC) for insect control, and molybdenum (0.35 g kg^{-1}). *Mesorhizobium ciceri* inoculant (Novozyme, Cambridge, MA) was applied to each seed packet (1 packet $block^{-1}$) one day before planting. Entries were planted at a density of 43 seeds m^{-2} in a 1.5 m × 6.1 m block. All yield trials used a randomized complete block design with three replications. Weeds were controlled by a single post-plant/pre-emergence application of metribuzin (0.42 kg ha^{-1}, Bayer Crop Science, Raleigh, NC) and linuron (1.34 kg ha^{-1}, NovaSource, Phoenix, AZ). No fungicides were applied to control Ascochyta blight. Plots were mechanically harvested using a research combine on August 29, 2013 at Dayton and on September 4, 2013 at Pullman WA.

2.2. Ground-truthing of seed size

Approximately 250 g samples of cleaned harvested seed from each plot was separated using a series of round holed screens that included a 4 mm screen on top followed by 9.9 mm, 9.1 mm, 7.9 mm screens and a catch pan. The seed samples were placed on top of the 9.9 mm screen and the 4 mm screen was placed on top of this to prevent loss of seed. The samples and separating screens were placed in a shaker (New Brunswick G2) and agitated at 100 rpm for 30 s. The amount of seed on top of each screen was weighed and converted to a percent of the weight of the total sample. The distribution of the size of seed for each plot was estimated as the percent of harvested seed that was >9.9, >9.1, >7.9 mm, or <7.9 mm.

2.3. Image acquisition

All the images were acquired in our laboratory at Washington State University, Pullman. A digital SLR camera (70D, Canon, Tokyo, Japan) was setup on a tripod with a 15–85 mm zoom lens attached. In order to keep image exposure consistent, all the images were captured at manual mode with 18 mm focus lens, aperture of f/4.5, shutter speed of 1/40 s, and ISO 250. The resolution was set to 2700 × 1800 pixels, which can provide contour details of the chickpeas. A coin was put aside to calibrate actual seed size during image processing. Fig. 1 shows the camera setup used for collecting 144 images, which represented 72 samples from each of the two locations.

2.4. Image processing and analysis

The seed images were analyzed with a macro developed using Image J (Schneider et al., 2012) to batch process all the images for estimating number of seeds and seed size. Following the analysis using Image J, Matlab® (R2009b, The MathWorks, Inc. MA), a computing language used for data processing and analysis, was used to summarize the results for better comparison with the ground-truth size distribution. The procedure of analysis is further explained.

Each image was converted into 8-bit gray scale image, where the pixel values are scaled linearly according to the image minimum and maximum, which is set to 0 and 255, respectively. A threshold of 60 was selected based on the experimental set-up to convert the image into binary image (0 and 255). Following binary conversion, watershed algorithm (Vincent and Soille, 1991; Roerdink and Meijster, 2000) was applied to segment the chickpeas seeds that were in contact with each other. The algorithm computes the Euclidian distance map and determines the ultimate eroded points (UEP), following which it expands the UEP until edges of particles are reached or it comes in connection with another UEP. To ensure the applicability of watershed for seed separation/segmentation, images with and without manually separated seed samples were analyzed. In separated seeds image, the seeds were not in contact with each other, while in non-separated seeds image, several seeds were in contact (connected) with each other. The results were compared with ground-truth data. It was observed that the correlation between the ground-truth seed size and size estimated using non-separated seed images were similar or better than that of images with separated seeds (Fig. 2). Thus, it was found that the watershed algorithm was applicable for seed separation.

Following this process, a scale was set prior to particle analysis. A quarter (U.S. coin) with a known diameter of 24.26 mm was used as a standard measure (scale). If the distance between the camera and seeds was constant, the number of pixels representing the diameter of coin could be used as a standard. The number of pixels representing coin diameter (24.26 mm) was estimated using Image J measure tool, and later added to the macro. Finally, the particle analysis was performed with a set measure between 20 to 150 mm to eliminate the background noise and coin. The circularity was set between 0 and 1. The Image J macro could batch process all the images from the desired folder and save the results in the target folder. The various steps and sample images are shown in Fig. 3.

Fig. 1. Image acquisition system set-up.

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