Image analysis of mustard seed: its utilization in assessing seed uniformity

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Abstract

Mustard millers have long had a strong interest in obtaining uniform seed. They prefer mustard seed to be all the same colour. Oriental and yellow mustard should be uniformly bright yellow and brown mustard should be dark brown. Admixtures of colors are not tolerated. Secondly, to ease milling difficulties the seed should be, as nearly as possible, all the same size and shape (like ball bearings). Reflective colorimeters can assess seed color and predict which seed sample is the brightest yellow or the darkest brown but they fall short in assessing how uniform the seed color is among seeds or within individual seeds (mottling). 1000 seed weight is used to estimate the average seed size of a sample, but it does not provide information on uniformity of seed size. To do this individual seed weights of many seeds per sample have to be recorded - a tedious and time-consuming task. Visual observation can provide a rough estimate of seed color, size and shape, but is a subjective measurement. With the advent of digital cameras and image processing software it is now possible to routinely assess seed uniformity. A Canon EOS Digital Rebel was utilized to photograph 300 seed samples of seed harvested from several locations and years of the Western Canada co-operative mustard trial. The photographs were then processed with the public domain program "ImageJ". The "Analyze Particles…" option was used to calculate seed size, shape and shade data for each seed of each sample which was used to generate size, shape and shade uniformity statistics. With this setup up to 200 samples could be photographed and processed each day per technician. It was found that there were consistent differences in seed uniformity among the different cultivars and lines in each category of mustard, suggesting that there were genetic differences between lines which could be capitalized on in future breeding efforts.

Key words: Mustard, *Brassica juncea*, digital image analysis, seed size, seed shape, seed shade, ImageJ

Introduction

The mustard trade has strict regulations with respect to cleanliness, allowing not more than 0.7% extraneous material and not more than 2% damaged or shrivelled seed (Hemingway 1995). The seed coat is a significant, variable portion of *Brassica juncea* and *Sinapis alba* seeds, and its proportion influences the economics of dry-milling where the seed coats are removed, and wet-milling of *B. juncea* where the seed coat is left in or a portion screened out. Seed shape and uniformity of size are important to maximizing seed coat separation/milling (Hemingway 1995, 1997). Also the tendency of seed coats to break into tiny fragments affects the quality of mustard (Hemingway 1995). Appearance is very important to the mustard, thus oriental mustard and yellow mustard seed should be a bright yellow with no dark seeds. Brown mustard seed should be dark brown in colour with no light coloured seeds. As our centre (Saskatoon Research Centre) is the primary breeding institution for condiment mustard, the Canadian Mustard Association has asked us to develop highly uniform cultivars of oriental, brown and yellow mustard in terms of seed size, seed shape (as round as possible) and seed colour. For breeding applications methods of analysis must be accurate and, importantly, rapid and it seemed that digital image analysis could be an ideal method to approach this problem. A digital camera and the free scientific image processing program, ImageJ (Abramoff et al. 2004, Rasband 1997-2006) are the essential tools to approach this task. Others have used image analysis to assess visual characteristics. Cober et al. 1997 studied the heritability of seed shape and size in soybeans, Dell'Aquilla 1997 and Ducournau et al. 2004 used image analysis to monitor germination of broccoli seeds and sunflowers, respectively, and Tanska et al. 2005 studied surface features and colour of rapeseed. Others have used image analysis for seed classification purposes (Utku et al. 1998, Wrigley 1999, Sako et al. 2001).

Material and Methods

Digital imaging hardware: The imaging hardware consists of a Canon EOS Digital Rebel camera (6 megapixel sensor; EF-S 18-55mm f/3,5-5,6 USM lens,), an IFF Mini-Repro copy stand with 4 lights and a transparency viewer (GEPE 8" x 12" Slimlight Illuminator). The camera is mounted on the copy stand post 30 cm above the slide viewer mounted on the base. The camera settings are auto focus, M, ISO 100, optical zoom 35, aperture 16, shutter 1/10, exposure 0 sharp 0, and white balance: Fluorescent. Images are captured in large/fine JPG format to a database of pictures (Microsoft Access). The camera settings are not changed throughout and a picture of a ruler is used to scale the seed sample images. Total cost of the equipment was less than $2000 Canadian.

Seed material and seed sample preparation: The seed material used for this study came from the Western Canada co-operative mustard trials conducted each year at several locations across the prairies. Seeds are sampled from harvest envelopes using a 300 seed vacuum seed counting head (Hoffman Manufacturing, Inc., Albany, OR, USA). They are placed on a 20 x 25 cm glass plate which has been lightly sprayed with Krylon® Easy-Tack™ repositionable spray adhesive by placing the seed head upside down and releasing the vacuum. The adhesive is essentially invisible to the camera and holds the seeds in place. Plastic film is positioned at the top and bottom of the glass plate such that it catches the top and bottom edge of the seed head and keeps it just slightly off the surface. The plate is positioned on the image viewer and a picture is taken and stored in the database. The seeds are removed by scraping them into a container using a straight edge and the plate is reused. The adhesive treatment typically lasts for about 50 – 200 images before it becomes so soiled that it interferes with image quality. The glue is easily washed off with soapy water and reapplied. In this manner about 100 to 200 seed sample images are created per day.

Image Processing: The particle analyzing option of the freeware image processing program, "ImageJ" (Rasband 1997-2006) is used to generate descriptive statistics for individual seeds of the sample and of the entire population. A small plugin (a modification of the Batch Converter plugin, Rasband 1997-2006) was created to allow automatic processing of images in a folder with generation of particle/seed statistics for each image. The images are converted to 8-bit greyscale, a threshold of 0-180 is applied and several statistics are generated for each seed in the image including area, mean grey value, standard deviation of grey value, perimeter, major and minor axis, circularity, Feret's diameter, etc. The batch program processes about 100 pictures in 5 minutes. This data is stored in the database where it can be manipulated as desired.

Results

A typical image of 300 seeds is shown in Fig. 1a. As the image size is 3072 x 2048 pixels each seed is represented by greater than 500 pixels. Different shapes, sizes and colours of seed can be readily seen. Also it is evident that some seed are broken and some are germinated. All seeds are cleanly separated which aids the software in discrimination. A comparison of Fig. 1a and 1c with their threshold counterparts (Fig. 1b and 1d) reveals that the threshold (red) closely follows the outlines of the individual seeds and completely covers the seed, so accurate estimates of seed size, shape and shade can be obtained.

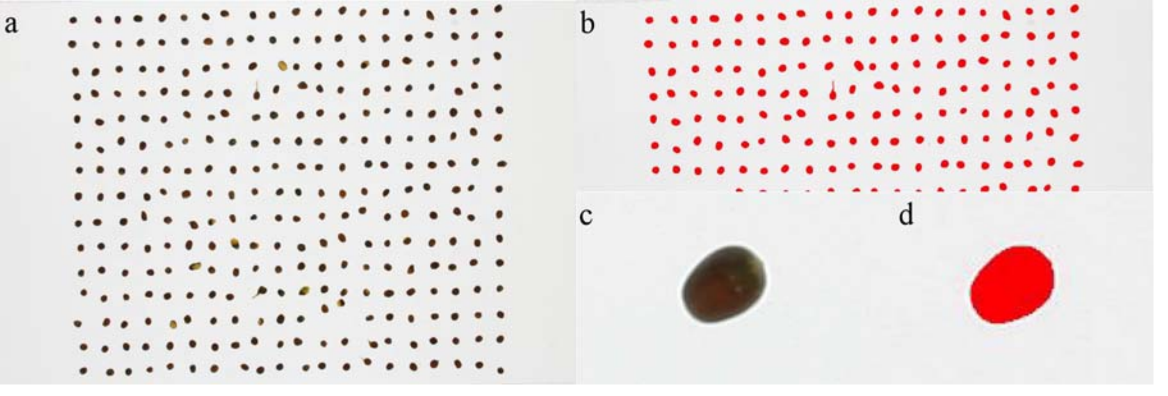

Fig.1 Image of 300 seeds of brown mustard, a line with poor seed quality (a: original picture; b: image top half, 8-bit greyscale conversion and threshold applied; c: a single seed; d: same seed, threshold applied).

Values for area, diameter and greyness generated for each seed can be averaged and compared to traditional methods estimating seed mass (1000 seed weight) and colour (whiteness index generated by a Hunterlab Miniscan colorimeter). Fig. 2a compares image analysis average area data with 1000 seed weight. A strong correlation was found (R^2 = 0.966). Fig. 2b demonstrates that diameter also correlates strongly with 1000 seed weight (R^2 = 0.962), as expected. Fig. 2c shows that average mean grey value can be used to estimate seed brightness, the same as the whiteness index.

Table 1 compares three oriental mustard entries in the 2005 cooperative mustard trial for seed characteristics that can be determined by digital image analysis. There is a consistent difference across locations in average seed area between Forge (a small seeded cultivar), AC Vulcan and J01-1429 (a line selected for increased seed mass). The standard deviation of area (uniformity of seed size) is also consistently higher in J01-1429 than Forge and AC Vulcan. In circularity (a measure of roundness) and in standard deviation of circularity Forge is consistently the closest to a round shape and has the least variation of shape (both desirable traits, Hemingway 1995, 1997). In mean grey value AC Vulcan is better than Forge and J01-1429. But there is no clear winner for uniformity of shade (standard deviation of mean grey value).

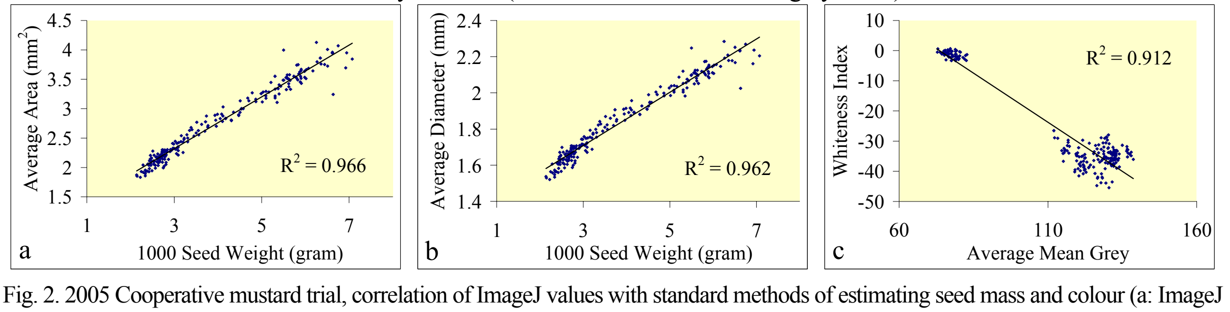

Fig. 2. 2005 Cooperative mustard trial, correlation of ImageJ values with standard methods of estimating seed mass and colour (a: ImageJ average area versus 1000 seed weight, b: ImageJ average diameter versus 1000 seed weight, c: ImageJ average grey value versus whiteness index).

Table 1: Seed uniformity comparisons of three oriental mustard entries in 2005 cooperative mustard trial

Entry	Location*							
	C1	E2	I0	R1	S1	S6	W1	Avg
Area (mm^2)								
Forge	1.90	2.08	2.12	2.05	2.15	1.82	2.10	2.04
AC Vulcan	2.07	2.33	2.27	2.31	2.38	2.01	2.28	2.24
J01-1429	2.71	2.86	2.71	2.80	3.00	2.56	2.85	2.79
SD Area (mm^2)								
Forge	0.30	0.38	0.38	0.35	0.35	0.34	0.37	0.35
AC Vulcan	0.28	0.37	0.34	0.41	0.35	0.33	0.34	0.35
J01-1429	0.51	0.50	0.42	0.52	0.54	0.45	0.48	0.49
Rel. SD Area (%)								
Forge	15.8	18.5	17.7	16.8	16.1	18.5	17.6	17.3
AC Vulcan	13.8	15.8	15.1	17.8	14.5	16.4	14.8	15.4
J01-1429	18.7	17.4	15.6	18.4	18.0	17.7	17.0	17.6
Circularity								
Forge	0.908	0.893	0.896	0.903	0.901	0.903	0.895	0.900
AC Vulcan	0.902	0.888	0.889	0.878	0.895	0.895	0.887	0.890
J01-1429	0.895	0.883	0.891	0.890	0.890	0.894	0.885	0.890
SD Circularity								
Forge	0.015	0.036	0.024	0.028	0.024	0.020	0.034	0.026
AC Vulcan	0.025	0.035	0.025	0.064	0.022	0.018	0.038	0.033
J01-1429	0.034	0.041	0.024	0.038	0.035	0.022	0.034	0.033
Mean Grey Value								
Forge	122.8	121.0	115.3	116.7	116.9	123.6	120.5	119.4
AC Vulcan	129.5	127.0	121.6	122.0	123.2	130.0	128.1	125.8
J01-1429	122.6	119.2	115.5	115.0	116.5	121.3	121.4	118.5
SD Mean Grey								
Forge	7.2	7.3	12.3	9.0	8.4	5.8	9.3	8.6
AC Vulcan	8.1	7.0	12.4	10.9	9.5	5.6	7.4	8.8
J01-1429	8.2	8.2	12.4	11.1	9.4	6.1	7.7	9.2

* Locations: (C1 – Congress AB, E2 – Eyebrow SK, I0 – Irricana AB, R1 – Rosebank MB, S1 – Saskatoon SK, S6 –Swift Current SK, W1 Weyburn SK), Avg – average of all locations, Area – average area, Circularity – (A measure of roundness, $4*pi*area/perimeter^2$, 1 is a circle, 0 is a line), Mean Grey Value – average greyness (a higher number is brighter), SD – standard deviation.

Discussion

The data for only three lines of oriental mustard in 2005 is shown. But co-operative mustard image data from 2002, 2003 and 2004 is available as well. The observations described here are consistent with the observations made in previous years. Forge is consistently the smallest, but is the roundest and the most uniform in terms of size and shape. AC Vulcan has consistently larger seed than Forge and is a brighter seed, but is not quite as round. Similar observations of differences in seed size, shape, colour and uniformity have been observed amongst cultivars of brown mustard and yellow mustard as well.

This method is also used to conduct selections within breeding populations of mustard and is useful for quantifying the susceptibility of certain lines to seed damage and early germination. These events drastically alter the shape of the seed and thus manifest themselves as a reduction in the circularity value. These events also drastically affect the color or mean gray value, as an emerging radical or the visible meat of a seed is significantly brighter than the brightest intact oriental mustard seed.

Conclusions

Digital image analysis is a useful tool for rapid screening of a large number of samples for seed size, shape and shade parameters including estimates of the uniformity of these seed characteristics. Small differences are detectable. The “ImageJ” software program is cost-effective (free), powerful and easily adaptable and is recommended for anyone on a budget. Different cultivars of mustard differ in their seed size, seed shape (some lines are more round or circular) and colour, but by this image analysis method it can be seen that different lines of mustard also differ with respect to uniformity of seed size and shape. The equipment and software needed to conduct digital image analysis is relatively inexpensive and many seed samples can be processed in a single day, up to 200.

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