USING DIGITAL IMAGE ANALYSIS TO ACCURATELY DETERMINE THE THOUSAND KERNEL WEIGHT OF RANDOMLY DISTRIBUTED BARLEY, MALT AND WHEAT SAMPLES

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Introduction

Rapid, accurate and non-subjective grain tests help growers and processors agree on a fair price and ensure that the grain is used for the most appropriate purpose. Common tests include protein, moisture, test (hectolitre) weight, screenings and dockages for pests, disease and foreign matter (Vicgrain, 2000). Each test contributes to a more comprehensive assessment of the grain. The tests assist breeders when selecting promising new cultivars.

Plant breeders and maltsters also use the thousand kernel weight (TKW) test, which provides additional information on seed morphology. The test indicates the average kernel weight, with the units expressed as grams per thousand seeds. TKWs are valuable to maltsters and millers as high TKW kernels are plumper, malt and/or mill more evenly and have a higher proportion of endosperm than small kernels. The high TKW grains also produce more attractive malt (Stuart, 1998). TKWs assist breeders in selecting large kernel cultivars and permit growers to calculate their optimum sowing rates (Schwarz and Horsley, 1995).

Handcounting the kernels for a TKW is tedious and time-consuming. Using a seed tray, which has indents to hold 100 kernels, speeds up the process and reduces the tedium, but still requires 10 to 15 minutes to count the standard 40 grams of seed for barley TKWs (Institute of Brewing, 1999). Laboratories that make frequent TKW determinations usually use electromechanical seed counters such as the Numigral or Countador counters.

Digital image analysis (DIA) can potentially count the kernels rapidly and accurately, but kernels touching others are difficult to count. Simply ignoring touching grains would result in inaccurate TKWs because only part of the sample mass would be used in the count. DIA systems have been developed that use conveyer belts (GrainCheck) or vacuum assisted trays (Maztech) to physically separate the seeds. These systems can work well, but the specialised hardware makes them very expensive.

Another approach to DIA is to use commonly available computers and flatbed scanners and develop an algorithm that will count all of the single and touching grains in a randomly distributed sample. Shatadal (1994) developed a shape recognition algorithm that digitally cut apart touching grains with 93% accuracy. We developed macros that use Scion Image's image edge erosion routines to separate the kernels with some success. Here we report the development of "SeedCount", which is based on a novel algorithm, for the counting of kernels. SeedCount is more rapid and precise than most of the above approaches.

DIA can be used for much more than simple TKWs. Many aspects of kernel morphology and cultivar identification have been studied using DIA (Gebhardt *et al*, 1993; Symons and Fulcher, 1987).

Materials and Methods

The winter wheat cultivars (Brennan, Gordon, Kellelac, Meering and Silver Star) were provided by Wrightson Research and Goodman Fielder Mills. Three subsamples were taken from each bulk sample. The subsamples were counted in duplicate for each method. Screened (>2.8 and <2.2 mm) sub-samples of Kellelac were included to test the limitations of the DIA software. Some barley and malt samples were also analysed.

Hand counting was performed with and without the seed trays. Electromechanical counting was performed on a Numigral 1 and a Kirby KL9 counter.

Scion Image is a freeware program provided by Scion Corporation (http://www.scioncorp.com/). Scion Image is the PC version of Image, a Macintosh program produced by the United States' National Institute of Health. Weiss Associates developed the SeedCount system with consultation from the University of Ballarat. SeedCount and Scion Image were run under Windows 98SE on a PC with a Pentium II 300mhz processor and 64 megabytes of memory and mainly used a Hewlett Packard Scanjet 5300C scanner.

Kernel samples of about 35 grams were weighed to the nearest 10 milligrams and distributed onto the scanner using a positioning frame. A coarse-toothed comb was used to spread the kernels and the frame gently shaken to even out the kernel distribution. Placing a black acrylic box over the glass window of the scanner provided a contrasting background. The scanner's TWAIN interface was called from SeedCount. The scan was automatically inverted, cropped (when using the Scan Tray) and loaded into SeedCount. The image can be saved, counted and the data appended to a file that can be easily incorporated into a spreadsheet.

All TKWs in this paper are calculated on a dry weight basis. Moisture determinations were made with the standard oven method (Institute of Brewing, 1999).

Results and Discussion

Our approach to the DIA multi-kernel cluster problem is unique. SeedCount detects and counts the single grains in the sample using an algorithm based on the patented MACE software (US Patent 6,243,486 B1 -Weiss Associates). MACE has been developed to count cells in histology and microbial colony forming units in petri dishes (http://www.colonycount.com). SeedCount calculates the average cross-sectional area of the single kernels and uses this value to determine the number of kernels in each of the multi-kernel clusters.

The graphs illustrate the accuracy and precision of the main methods tested. Figure 1 shows the accuracy of TKWs calculated for wheat samples handcounted using seed trays. The X and Y axes are the initial (A) and replicate (B) TKWs. The "ideal" line on all of the graphs matches "perfect" TKWs where the initial (X value) and replicate (Y value) TKW are identical. The correlation (r=0.99996) and Standard Error of Estimate (0.07) show that the Hand plus Tray counts are very accurate. Figures 2 and 3 respectively show the results for wheat for the electromechanical and DIA (SeedCount) TKWs versus the average Hand plus Tray count. A and B are the two replicate counts. It can be readily seen by the close fit to the ideal line that SeedCount (Figure 3; r=0.9992, SEE=0.25) is more accurate and precise than the electromechanical counter (Figure 2; r=0.984, SEE=1.15). Trials demonstrate that SeedCount is able to accurately count wheat samples ranging from 40 to 1700 seeds.

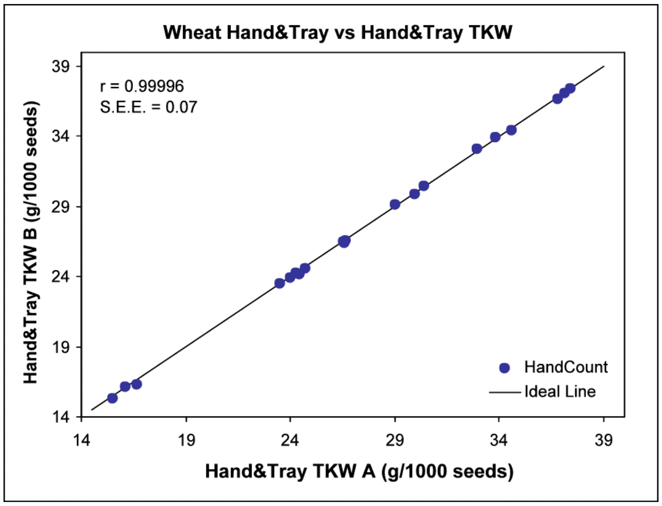

Figure 1: Comparison of Replicate TKW values determined by Handcounting using Seed Trays.

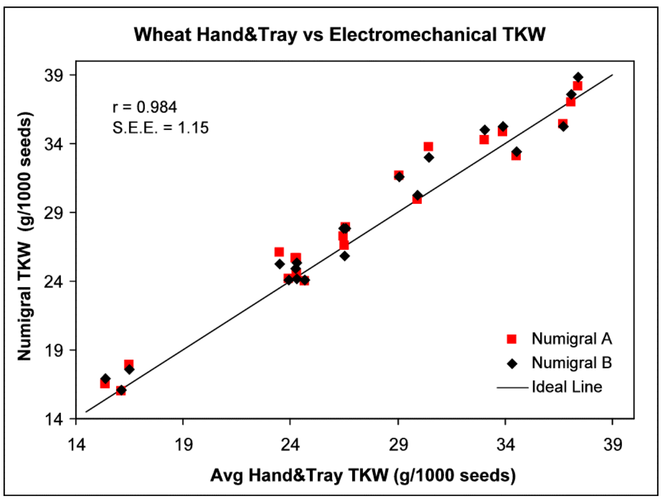

Figure 2: Comparison of Replicate TKW values determined by Electromechanical counters versus the Average Handcounting using Seed Trays values.

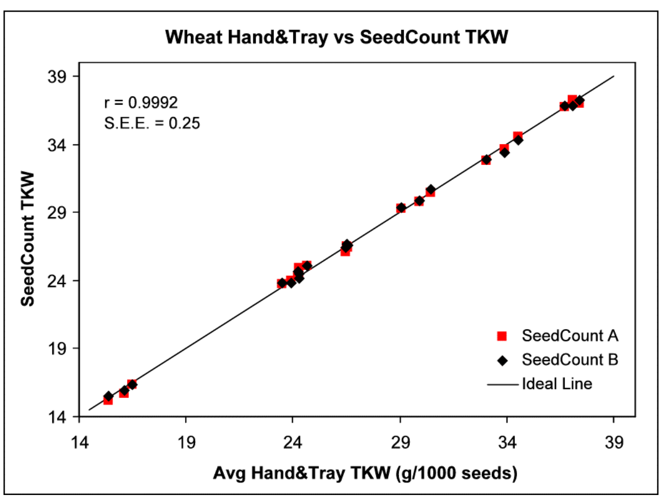

Figure 3: Comparison of Replicate TKW values determined by SeedCount versus the Average Handcounting using Seed Trays values.

Scion Image erosion counts were hampered by image erosion problems. Some eroded kernels remained connected together while other kernels were cut into several pieces. This made accurate counts (and therefore accurate TKWs) very difficult with this method.

The parameters summarised in Table 1 are combined results for wheat, barley and malt. The Hand plus Tray method has the highest correlation (1.0000) and lowest standard error of the estimate (0.065), but it is the second slowest method to use at 12.5 minutes per sample. SeedCount (r=0.9993, SEE=0.306) and careful hand counting (r=0.9995, SEE=0.299) have similar accuracy. SeedCount is clearly the fastest counting method (0.9 minutes per sample including scanning). The electromechanical counters, Kirby and Numigral, had usable accuracy (r= 0.9987 and 0.990, SEE=0.459 and 1.186 respectively), but were quite slow (6.6 and 8.6 minutes respectively) compared with SeedCount. The Scion Image erosion TKW method had inadequate accuracy (r=0.906, SEE=2.5) and was much slower (6.4 minutes per sample) than the SeedCount method.

Table 1: Comparison of Counting Methods

Method	Correlation	Std Error	Speed (min)
Hand plus Tray	1.0000	0.065	12.5
Hand Only	0.9995	0.299	18
SeedCount (DIA)	***0.9993***	***0.306***	***0.9***
Kirby (EM)	0.9987	0.459	6.6
Numigral (EM)	0.9900	1.186	8.6
Scion Image (DIA)	0.9058	2.495	6.4

Digital Image Analysis, run on computer equipment found in most laboratories, is capable of determining precise TKWs. Initial results and work by others suggest that DIA has the potential to generate accurate screenings equivalents (Kuhbauch and Bestajovsky, 1989).

Conclusions

Handcounting assisted with seed trays is the most accurate counting method for performing TKWs. Electromechanical counters provided acceptable accuracy at a slow counting rate.

DIA can be relied on to calculate TKWs. SeedCount's unique algorithm provided fast and accurate TKWs at a reasonable cost.

Acknowledgements

This research was made possible with funding from the Australian Research Council and Wrightson Research.

The gracious assistance of Samantha Openshaw (Agrifood Technology), Jarrod Gooden and Alex Severino (Joe White Maltings) and Paul Panozzo (Goodman Fielder Mills) is also gratefully acknowledged.

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