Indian J. Genet. Plant Breed., (2023); 83(4): 469-475
https://doi.org/10.31742/ISGPB.83.4.2
ISSN: 0975-6906
www.isgpb.org

RESEARCH ARTICLE

Development of a novel image-based grain counting setup for thousand-grain weight estimation in wheat

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Abstract

Thousand-grain weight (TGW) is one of the major yield-contributing traits routinely used as a selection criterion by plant breeders. It is also an important grain quality trait that determines milling yield. Accurate phenotyping of TGW is imperative to dissect its genetics for yield improvement. The traditional approach to TGW estimation involves manual grain counting and weighing, which is laborious, tedious and less accurate for large sample sizes. As an alternative, we propose a customized grain counting setup for accurate estimation of TGW in wheat by assembling a photo lighting tent and a smartphone for image acquisition of grain samples. A popular open-source software, 'imageJ' was used to process the images to estimate the grain count. The counted grain samples were weighed to calculate the TGW. The TGW estimate derived from the proposed grain counting setup displayed a high degree of correlation with the manually estimated TGW data ($r = 0.99$, $p < 0.05$). It took significantly less time to count the grain samples using the proposed setup compared to manual counting with better accuracy and minimal labor. The error rate in grain counting using the imaging-based setup was very low (<1%) and 30 to 40 grain samples can be imaged per hour. This setup can be extended to estimate the TGW of different crops, excluding those having spherical seeds.

Keywords: Thousand grain weight, imaging, grain counting, imageJ, wheat.

Introduction

Wheat is an important cereal crop consumed across the globe and its demand is increasing day by day (Shewry and Hey 2015). To keep pace with the expanding global population, food production need to increase by 50% before 2050 (FAO 2017). Thousand-grain weight (TGW) is one of the principal components affecting grain yield, the rest being grain number per ear and productive tillers per unit area (Surek and Beser 2003; Yousaf et al. 2017; Ullah et al. 2021; Farokhzadeh et al. 2023). Thousand-grain weight is reported to be positively correlated with grain size, grain length, grain width, grain area and grain yield (Dholakia et al. 2003; Abdipour et al. 2016; Sefaoglu 2023). Seeds with higher TGW tend to have better vigor and germination rates (Ambika et al. 2014). Larger grains provide more nutrients to the developing embryos and help in proper root development and seedling establishment (Zohaib et al. 2018; Muhsin et al. 2021). Plump and bold grains are preferred by grain mills and consumers of certain geographies (Ponce-Garcia et al. 2017; Custodio et al. 2019). Being a quantitative trait, TGW is controlled by multiple genes and warrants the use of quantitative trait loci (QTL) analysis to identify the genomic regions controlling it (Sun et al. 2009; Li et al. 2015; Jha et al. 2022). Accurate phenotyping of TGW is crucial for capturing the phenotypic variation in the mapping populations with the minimum error to aid in QTL discovery. The traditional phenotyping method involves manual counting of 50 to

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How to cite this article: Krishnan J.N., Kumar U., Kaur S. and Chhuneja P. 2023. Development of a novel imaging-based grain counting setup for thousand-grain weight estimation in wheat. Indian J. Genet. Plant Breed., **83**(4): 469-475.
Source of support: Department of Biotechnology, Ministry of Science and Technology, Govt. of India.
Conflict of interest: None.
Received: April 2023 **Revised:** Aug. 2023 **Accepted:** Oct. 2023

300 grains, weighing and calculating TGW (Su et al. 2011; Zhang et al. 2013). The traditional method is laborious, time-consuming and less accurate for large sample sizes. Its efficiency varies based on the skill level of the worker, the number of grain samples and grain sample size. As an alternative, image acquisition and processing can be deployed to improve the accuracy and speed of grain counting (Paige et al. 1991; Sabanci et al. 2016).

A number of software and instruments have been developed for grain counting and thousand-grain weight estimation. 'GainTKW', an android-based mobile application was developed for the estimation of grain weight by integrating grain weighing and counting (Wu et al. 2018). Similar mobile application-based grain counting methods have been developed recently that can distinguish closely spaced grains as well (Komyshev et al. 2017; Liu et al. 2017; Gao et al. 2017). Sabanci et al. (2016) used Matlab software to process the images of the wheat grain samples spread on a dark background for grain counting, followed by grain weight estimation. An image analysis-based protocol for grain counting in maize was devised by Makanza et al. (2018) that involves processing photographed grain images using open-source software imageJ. Singh et al. (2019) implemented a machine-learning model on processed images to predict the size and mass of rice grains. A Python-based software package with a graphical user interface, 'Gridfree' was developed by Huand Zhang (2021) that uses an unsupervised machine learning approach, K-means, to measure grain count, area, length and width. Instruments and machines are available on the market for estimating grain count and weight but are expensive, time-consuming and specific to certain classes of seeds. There is a need to develop a technology-driven, rapid, low-cost and user-friendly methodology for precision phenotyping of thousand grain weight. To cater to this need, we propose an ingenious grain counting setup for estimating the thousand-grain weight of wheat based on imaging and image processing.

Materials and methods

Seed materials and manual grain counting

The grains of the ten wheat varieties released at the state/national level were used to test the efficiency of the grain counting setup. The ten wheat varieties included six bread wheat cultivars (HD2967, HD3086, DBW17, WH1105, PBW723 and PBW725) and four durum wheat cultivars (PBW114, PDW233, PDW274 and PDW291). Each variety was replicated thrice, and ten grams of grain were sampled per replicate. In the case of manual grain counting, the seeds were counted by spreading them in small trays with dimensions of 22 cm by 27 cm by 6 cm (Length, Width, Height). The grain count data was recorded.

Grain counting setup

A photo lighting light tent with dimensions of 40 x 40 x 40 cm was purchased from Amazon for about INR 4000 ($50) (Brand: House of Quirk). The tent comprises a cubical box with two LED panels for uniform illumination. The LED panels are provided with plugs for connecting to an external power source. A circular hole is provided on the top face of the cubical box for image capture from the top view, and the front face can be opened for image capture from the side view. The stage of the light tent was pasted with a white-colored plastic sheet for spreading the grain samples. A picture of the photo lighting light tent is given in Supplementary Fig. 1.

Image capturing

Ten grams of grain samples were spread widely and imaged so that each grain was selected as a distinct entity by the image analysis software. Photos of the spread grains were captured from the top angle at a fixed height using a Lenovo K8 Plus smartphone (13-megapixel camera). For each genotype, cardboard labels were placed near the borders of the stage for identification. The image dimensions were 4160 x 3120 pixels. Two people were involved in the imaging process, one for spreading the grain samples and the other for capturing images. After imaging all the varieties, the captured images were transferred to a laptop for further image processing. The methodology involved in image capture is schematically explained in Fig. 1.

Image analysis

The grain images were processed using the open-source software ImageJ (Schneider et al. 2012) (Supplementary Fig. 2). The image file was opened by clicking the 'Open' option from the 'File' menu. The opened image is first converted to 8-bit grayscale image by clicking the 'Image' menu followed by 'Type' and selecting '8-bit'. The image thresholding was done by choosing the 'Image' menu, followed by the 'Adjust' option, followed by the 'Threshold' option. This step converts the grayscale image into a binary image and the background contrast can be adjusted if needed. Alternatively, a binary image can also be generated by selecting the 'Process' menu followed by the 'Binary' option and selecting 'Make Binary'. The area containing the spread grains is selected from the binary image using the rectangular selection tool from the toolbar. The selected region is subjected to grain counting by clicking the 'Analyze' menu and choosing the 'Analyze

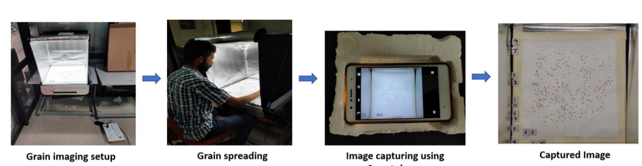

Fig. 1. Schematic outline of the steps involved in grain image capturing

Particles' option. A new dialog box opens, containing various parameters and options. In the 'Size' parameter, the value (in square pixels) was fixed as 200-infinity. This refers to the grain area cut-off value above which most of the wheat grains fall. Objects having a size of less than 200 square pixels are excluded from counting, and these can be broken grains, dust particles, husk, etc. This cutoff value can be fixed by observing the individual grain areas of unbroken grains calculated from different wheat varieties. In the 'Show' drop-down box, the 'Outlines' option is selected, which numbers all the individual grains from the selected region. The other parameters are set as default. The 'Summarize' box is checked and the rest of the boxes are left unchecked. A new 'Results' dialog box opens, displaying the average grain area of the processed sample along with the grain count data in tabular format. The 'Display results' box can be checked to tabulate the grain area of each grain belonging to the selected region (Supplementary Fig. 3). This table can be used to fix the minimum grain size cut-off value. The above steps can be repeated for each image, and the grain count data gets added one below the other in the 'Summary' table. These results can be directly copied and pasted into Microsoft Excel. A brief flowchart describing the image processing steps is given in Fig. 2.

Thousand-grain weight estimation

All the grains counted through image analysis or manually were weighed on the weighing balance (model: Citizen CY220, Aczet Private Limited, Mumbai). The thousand-grain weight (TGW) was calculated using the following formula in MS Excel,

$$TGW = [\text{Grain weight (in grams)}/\text{Grain count}] \times 1000$$

Grain count error rate calculation

The grain count error rate was used to predict the grain counting accuracy of the proposed imaging-based method. The error rate observed in grain counting by the imaging-based method in relation to the manual labor-based method (ground truth) is calculated using the following formula in MS Excel,

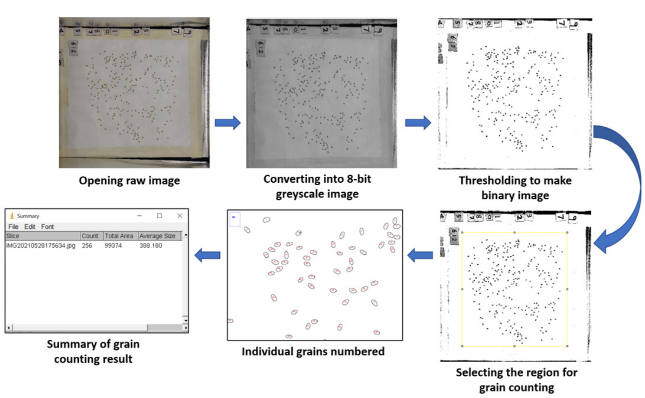

Fig. 2. Flow chart depicting the image processing methodology for estimating grain count using imageJ software

$$\text{Error rate (\%)} = [(N-N_0)/N] \times 100$$

where, N_0= mean grain count data of a variety calculated by the imaging-based method

N = mean grain count data of same variety obtained from manual counting

Statistical data analysis

The mean TGW and standard deviation values for each variety were calculated in MS Excel. The summary statistics and t-test of the TGW estimates were computed using PAST 4.03 software (Hammer et al. 2001). The correlation scatterplots and the box plots were computed using the 'ggplot2' package in R software (R Core Team 2013; Wickham 2016).

Results

Grain counting

The mean grain count data of the selected varieties is presented in Table 1. The estimates from both imaging-based and manual labor-based methods were nearly similar, and a t-test revealed the mean differences to be statistically

Table 1. Comparison of the mean grain count and thousand grain weight values of different varieties estimated by imaging-based and manual labour-based methods

Variety	Method	Grain count	Grain count Error rate (%)	TGW (g)
HD2967	Imaging-based	294.7 ± 7.6^{a}	0.20	33.9 ± 0.9^{a}
	Manual labour	295.3 ± 8.5^{a}		33.9 ± 1.0^{a}
HD3086	Imaging-based	319.0 ± 5.0^{a}	0.85	31.3 ± 0.5^{a}
	Manual labour	316.3 ± 6.1^{a}		31.6 ± 0.6^{a}
DBW17	Imaging-based	309.0 ± 5.2^{a}	0.42	32.4 ± 0.5^{a}
	Manual labour	307.7 ± 6.4^{a}		32.5 ± 0.7^{a}
WH1105	Imaging-based	270.0 ± 9.5^{a}	0.48	37.1 ± 1.3^{a}
	Manual labour	268.7 ± 10.5^{a}		37.2 ± 1.5^{a}
PBW723	Imaging-based	233.0 ± 3.6^{a}	0.43	42.9 ± 0.7^{a}
	Manual labour	232.0 ± 4.3^{a}		43.1 ± 0.8^{a}
PBW725	Imaging-based	248.7 ± 6.4^{a}	0.16	40.2 ± 1.0^{a}
	Manual labour	248.3 ± 5.8^{a}		40.3 ± 0.9^{a}
PBW114	Imaging-based	253.3 ± 7.8^{a}	0.00	39.5 ± 1.2^{a}
	Manual labour	253.3 ± 5.0^{a}		39.5 ± 0.8^{a}
PDW233	Imaging-based	231.7 ± 6.4^{a}	0.61	43.2 ± 1.2^{a}
	Manual labour	230.3 ± 7.6^{a}		43.4 ± 1.4^{a}
PDW274	Imaging-based	289.0 ± 10.5^{a}	1.15	34.6 ± 1.2^{a}
	Manual labour	285.7 ± 13.4^{a}		35.0 ± 1.6^{a}
PDW291	Imaging-based	256.7 ± 3.5^{a}	0.27	39.0 ± 0.5^{a}
	Manual labour	256.0 ± 2.6^{a}		39.0 ± 0.4^{a}

*Grain count and TGW values are represented as Mean ± Standard deviation

*Mean values labelled with different superscripts are significantly different at p-value<0.05 for Grain count and TGW

Table 2. Comparison of the summary statistics of the replicated data for thousand grain weight and grain count estimates obtained from imaging-based and manual labour-based methods

Statistics	Grain count (Imaging-based)	Grain count (Manual labour)	TGW (g) (Imaging-based)	TGW (g) (Manual labour)
Minimum	227	225	31.0	30.9
Maximum	324	323	44.4	44.0
Mean	270.5	269.4	37.6	37.4
Standard deviation	30.4	30.2	4.1	4.1

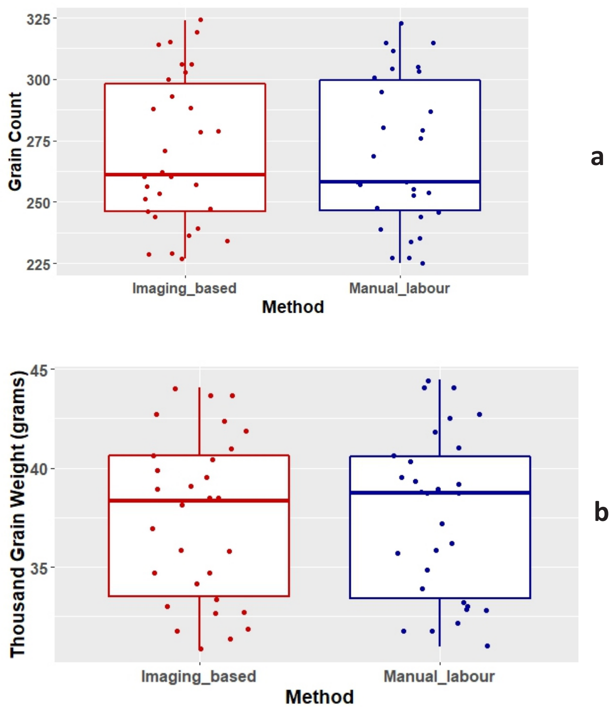

Fig. 3. Distribution of grain count (A) and thousand grain weight (B) values estimated by imaging-based and manual labour-based methods displayed in the form of boxplots overlaid with jitters

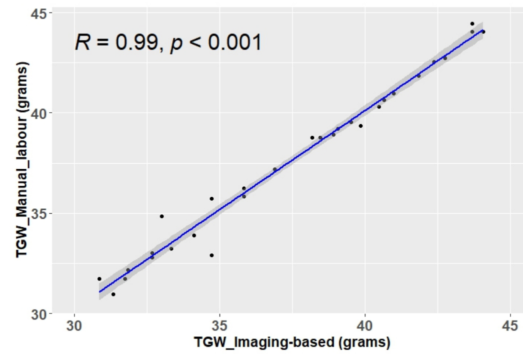

Fig. 4. Scatterplot depicting the degree of correlation between the thousand grain estimates derived from imaging-based and manual grain counting methods

non-significant. The summary statistics of the grain count data also revealed a similar picture (Table 2). From Table 2, it was observed that the mean grain count data calculated using the imaging-based method (270.5) was slightly higher than the manual labor-based method (269.4). The minimum and maximum values of the grain count estimated by the imaging-based method were slightly higher compared to the manual method. The lowest grain count was recorded for the durum wheat variety PDW233 (231.7 and 230.3), whereas the bread wheat variety HD3086 recorded the highest grain count (319.0 and 316.3) under both methods (Table 1). The error rates observed in grain counting by the imaging-based method in regard to manual counting were very low, ranging from 0 to 1.15% among the selected varieties (Table 1). This indicates that the imaging-based method has high accuracy. The distributions of grain count data obtained from imaging-based and manual labor-based methods are displayed in the form of box plots with grain count values jittered on them (Fig. 3A). Both the distributions had similar patterns without much deviation, indicating the accuracy of the imaging-based method with the ground truth values. Moreover, it took, on average, five minutes to count a single grain sample of 10 grams using manual labor. On the contrary, it took just around two minutes to spread and image the grain sample using the newly proposed imaging setup and an additional one minute to process the image for grain count estimation.

Thousand-grain weight estimation

The mean TGW values of the varieties are listed in Table 1, which indicates a high degree of correlation among the TGW estimates derived from imaging-based and manual labor-based approaches. This was further validated by a t-test that revealed the mean differences of TGW values calculated using both methods to be statistically non-significant. The durum wheat cultivar PDW233 recorded the highest TGW under both estimation methods (43.2 and 43.4 grams) owing to its lowest grain count among the selected varieties. This was obvious, as large-sized grains contributed to higher grain weight. Similarly, the variety with the highest grain count, cv. HD3086, recorded the lowest TGW (31.3 and 31.6 grams) under both methods. The distributions of the TGW values estimated from both methods were nearly identical, and the median TGW value of the manual labor-based method was slightly greater than the imaging-based method (Fig. 3B).

Correlation between TGW estimates of imaging-based and manual labor-based methods

The scatterplot depicting the extent of correlation between the replicated TGW estimates of imaging-based and manual labor-based approaches is given in Fig. 4. It is clearly evident that there is a high degree of correlation between the TGW estimates derived from the two methods (r=0.99; strong, positive correlation), as most of the data points fall on the trend line (line of best fit) or are located very close to the trend line.

Discussion

Thousand-grain weight (TGW) is one of the key traits contributing to grain yield, and an accurate estimation of TGW is crucial for carrying out selections in a breeding population and for conducting QTL mapping studies. The traditional approach to TGW estimation involves manual counting of a representative number of grains followed by weighing to calculate TGW. This method is tedious and laborious and its accuracy varies depending on the skill level of the worker and the sample size. Automated seed counting machines are available on the market, but are expensive and specific to certain categories of seeds. To overcome these limitations, image-processing-based methods have been developed in the recent past based on the Android operating system (Liu et al. 2017; Gao et al. 2017; Wu et al. 2018) and desktop PCs (Sabanci et al. 2016; Hu and Zhang 2021). In the present study, we developed an imaging-based grain counting setup for wheat that involves a photo lighting tent and a smartphone for grain imaging, followed by image analysis using imageJ software on a laptop or PC. The grains were spread manually on a white background ensuring that the individual grains do not touch each other. This step significantly reduces the error rate in grain count estimates, resulting in improved accuracy. The error rate in our grain count estimates was well below one percent in most of the grain samples (Table 1). It took on average 3 minutes to process a grain sample image using our method in comparison to other software (Gao et al. 2017; Liu et al. 2017; Wu et al. 2018) which consumed less than a minute. This limitation of our setup was offset by increased grain counting accuracy. Some of the reported software used different image segmentation methods to count grain samples that were not spread manually (containing a large number of touching grains) and that resulted in high error rates when sample sizes increased (Gao et al. 2017; Wu et al. 2018; Hu and Zhang 2021). We successfully demonstrated the counting of grain samples of selected wheat varieties with an average sample size of 270 grains, which is a good representation of the thousand-grain count. Background illumination is a major factor affecting the quality of smartphone-captured images, as demonstrated by the studies of McCracken and Yoon (2016) and Komyshev et al. (2017). In the current study, two LED strips were assembled inside the photo lighting tent that provided uniform illumination and resulted in high-quality shadow-free images (Supplementary Fig. 4). Looking at Table 1, we could find that the mean grain count values of the two varieties, HD3086 and PDW274, determined by manual counting were lower than the imaging-based estimates by 2 to 3 units. This may be due to human error during the manual counting of grains, as the imageJ software does not miss out on the selected grains. The 'size' option in the 'Analyze particles' function helps to exclude dust, dirt and broken kernels during the grain counting process. This helps to increase the precision of the grain count estimate. Using the proposed grain counting setup, we were able to process 30 to 40 samples per hour, depending on the competence of the workers. In contrast, the grain counting rate of manual counting was merely 12 to 15 samples per hour, which denotes the superiority of the proposed setup.

The grain counting setup can be extended to count grains of other crops having non-spherical seeds, including rice, maize, oats, barley, cotton, groundnut, soybean, gourds, pulses, etc. The setup is portable and can be folded and carried easily (Supplementary Fig. 1). It works decently with entry-level smartphones or cameras having a pixel quality of 12 megapixels or above. Photo lighting tents manufactured by various companies or brands can be used for assembling the grain counting setup. By replicating the imaging setup and manpower, the rate of grain samples processed per day can be doubled.

To summarize, we have proposed a cost-effective novel imaging-based grain counting setup for estimating the thousand-grain weight of wheat and other crops that is quick, cost-effective, less laborious and accurate. We tested the efficiency of the grain counting setup using ten different wheat varieties and compared the results to the ground-truth (manual labor-based grain counting and weighing) experiments. The thousand-grain weight data estimated using the imaging setup was highly correlated with the TGW estimates obtained from the manual labor-based method and, at the same time, is relatively less tedious and more precise. After validation on 10 wheat genotypes, the proposed setup was used for phenotyping a set of more than 300 advanced backcross introgression lines, and the data was used for QTL mapping for TGW, which is being reported in a separate manuscript.

Supplementary materials

Supplementary Figs. 1-4 are provided online, www.isgpb.org

Authors' contributions

Conceptualization of research (PC, JNK, UK); Designing of the experiments (JNK, PC); Contribution of experimental materials (SK, PC); Execution of field/lab experiments and data collection (JNK); Analysis of data and interpretation (JNK, PC); Preparation of the manuscript (JNK, PC).

Acknowledgments

The financial support provided by the Department of

Biotechnology (DBT), Ministry of Science and Technology, Government of India in the form of DBT Programme Support is gratefully acknowledged. The first author, J. Navaneetha Krishnan thankfully acknowledges the financial assistance provided by DBT, Government of India in the form of a Junior and Senior Research Fellowship during his doctoral studies.

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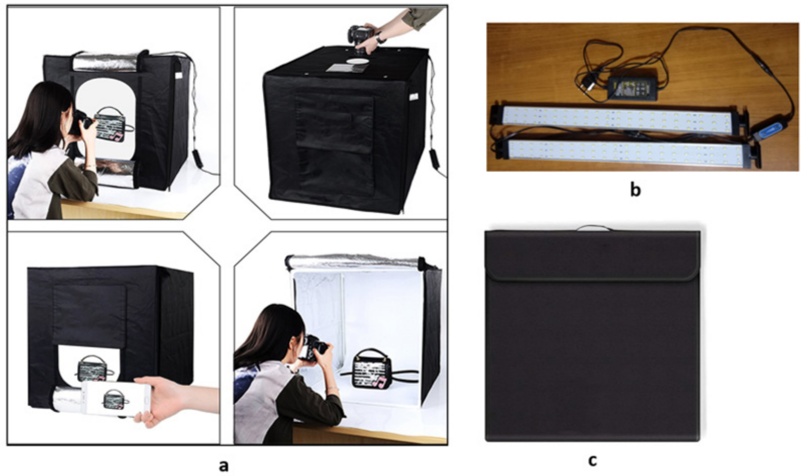
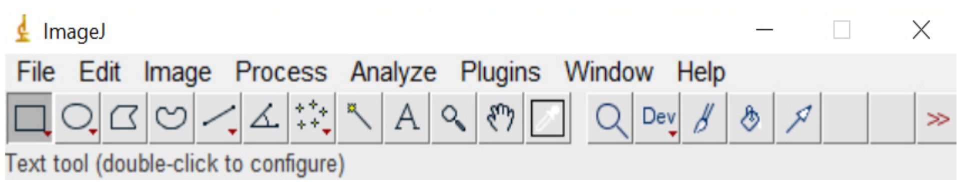


Supplementary Fig. 1. Photo lightingtent used for grain imaging: (a) 'House of Quirk' Photo lighting tent, (b) LED light panels for illumination and (c) Packed photo lighting tent is retrieved from: https://www.amazon.in/House-Quirk-Portable-PhotographyBackdrops/dp/B08LCBB45J)



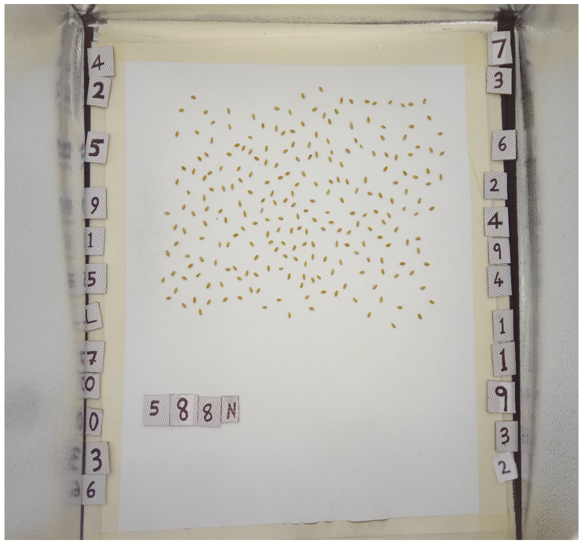
Supplementary Fig. 2. A screenshot of the ImageJ software version 1.52a

Results

File Edit Font Results

	Area	Mean	Min	Max
1	518	255	255	255
2	417	255	255	255
3	420	255	255	255
4	505	255	255	255
5	439	255	255	255
6	445	255	255	255
7	368	255	255	255
8	449	255	255	255
9	442	255	255	255
10	473	255	255	255
11	397	255	255	255
12	314	255	255	255
13	418	255	255	255
14	402	255	255	255
15	378	255	255	255
16	419	255	255	255
17	344	255	255	255
18	457	255	255	255
19	361	255	255	255
20	437	255	255	255
21	470	255	255	255
22	287	255	255	255
23	382	255	255	255
24	420	255	255	255
25	300	255	255	255
26	313	255	255	255
27	347	255	255	255
28	329	255	255	255
29	398	255	255	255
30	371	255	255	255

Supplementary Fig. 3. Results table displaying the grain areas of the numbered grains from aprocessedgra in sample image



Supplementary Fig. 4. Wheat grain sample image captured using the smartphone Lenovo K8 plus