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MACGRAN-IJ: AN EFFICIENT METHOD TO ANALYZE DIMENSIONS AND COLOR OF WHEAT GRAINS

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ABSTRACT

The dimensions, shape, and color of wheat grain are important in plant breeding, although their manual measurement is difficult and time-consuming. While it is true that digital image analysis (DIA) with specific software has made it easier, some are semiautomatic, exclude color, and require the digitization of individual grains. The goal of this study was to compare the precision, accuracy, and efficiency of MacGran-IJ versus SmartGrain, GrainScan, and manual caliper measurement. The morphological characteristics of durum wheat (*Triticum durum* Desf.) and bread wheat (*Triticum aestivum* L.) grains were measured using DIA with MacGran-IJ, including color (in RGB and CIELab color spaces), size (mm^2), lateral length (LLG, mm), ventral length (VLG, mm), lateral (LWG, mm) and ventral (VWG, mm) width, perimeter (mm), and circularity. Length, LWG, and VWG were measured with a digital caliper. The time (s) taken in each method was recorded. Size, LLG, VLG, and VWG were the same between methods for both species, but the LWG measurement obtained with vernier showed differences between the three programs ($p \leq 0.01$), as well as low accuracy and precision. VWG obtained manually had R^2 values of 0.92, 0.91, and 0.89 ($p \leq 0.01$) for MacGran-IJ, SmartGrain, and GrainScan, respectively. The ventral and lateral color red of wheat grain differed between MacGran-IJ and GrainScan; the lateral part of the bread wheat grain showed differences for red, green, and blue ($p \leq 0.05$). DIA in CIELab color required 11.57 s, and in RGB it needed 7.25 s ($p \leq 0.01$). Manual measurement of 50 wheat grains required 1528.07 s, whereas SmartGrain took 101.84 s, MacGran-IJ 45.4 s, and GrainScan 54.11 s (H = 36.59, $p \leq 0.01$). MacGran-IJ is an automated, simple, and efficient method for accurately measuring the size, length, and width of wheat grains using digitized images.

Keywords: *Triticum durum* Desf., *Triticum aestivum* L., digital images, accuracy, efficiency, grain morphology.

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INTRODUCTION

Wheat grain size, shape, and color are important traits for this cereal and must be considered in wheat breeding programs, since they influence yield, quality (Alemu *et al*., 2020) and commercial value (Sharma *et al*., 2021), and they can help to identify and classify varieties (Zhang and Ji, 2019). Wheat grain size and shape are associated to weight (Abdipour *et al*., 2016) and milling quality (Yan *et al*., 2017), as well as germination and initial seedling vigor.

The wheat grain color helps to evaluate grain purity (Bao *et al*., 2019) and healthiness (Goriewa-Duba *et al*., 2018; Ropelewska, 2019), so it is useful in classifying its quality (Velesaca *et al*., 2021). In addition, grain color is related to seed dormancy and consumer preference (Whan *et al*., 2014). Despite its importance, the grain morphology is largely unexplored, possibly because measuring its shape and size is difficult (Abdipour *et al*., 2016), even when performed with digital calipers.

Manual grain size measurement is commonly limited to length and width, thus resulting in low accuracy and error-prone data (Zhu *et al*., 2021). Another disadvantage of the manual technique is its limited capability in terms of the amount, quality, and variability of data available; it requires a large number of people to collect data, it is tedious, and it restricts sample sizes (Abdipour *et al*., 2016; Jamil *et al*., 2017). Visual measurements of attributes such as shape and color visually may be quick, but they are subjective and influenced by human and environmental factors. On the other hand, colorimetric analysis of wheat grain based on the CIELab color space requires expensive analytical equipment, although its readings are related to the pigment content of the grain and to the reflectance registered with digital images (Whan *et al*., 2014).

In contrast, measuring wheat grain morphology and color using digital image analysis (DIA) is a consistent, efficient, and objective technique (Goriewa-Duba *et al*., 2018). According to the International Union for the Protection of New Varieties of Plants (UPOV, 2013), DIA is an underused method for describing plant varieties and performing distinction, homogeneity and stability tests (DHE). The DIA technique is then recommended by UPOV as a replacement for visual evaluation of plant organs. Although visual evaluation is more widely used for economic, simplicity, and speed reasons, it is convenient to replace it with an efficient and inexpensive DIA using free software such as SmartGrain and GrainScan, or ImageJ (Whan *et al*., 2014). The first two software systems require user intervention and only work on Windows® OS, whereas ImageJ (https://imagej.nih.gov/ij/download.html) is multiplatform, open source, written in Java® and, while not specialized on grain measurements, it does allow automation performed via macros and plugins.

Automating the DIA process to characterize grain morphology reduces user intervention, favors repetitiveness, accuracy, and efficiency of measurements, and is user-friendly, even for inexperienced DIA users. The importance of quantifying wheat grain attributes for genetic, physiological, and plant breeding studies motivated the development of MacGran-IJ, which is presented here as a DIA-based, simple, and user-friendly method. The hypothesis is that MacGran-IJ is an efficient and accurate automated method for measuring both morphological and color variables of wheat grains. Therefore, this study was carried out to evaluate the accuracy, precision, and efficiency of the proposed MacGran-IJ method, as well as to compare it with the SmartGrain and GrainScan methods, in addition to manual measurements performed with a digital caliper.

MATERIALS AND METHODS

The MacGran-IJ method described here utilizes a routine or macro developed in the processing and image analysis ImageJ software ver. 1.53k, which is available for use on the Windows®, Linux and Mac OS® operating systems. The routine designed here helps the automated grain measurement and is implemented as a button on the toolbar, with the option of processing individual images or all those contained in a folder (batch processing files). Measuring wheat grains requires color scanned images in jpeg or other formats (tiff, bmp, pgm, png) with grains that could be touching each other on black background.

The automated MacGran-IJ set a scale based on the image resolution, is able to individualize each grain even when touching others and, for each one, it may register its size in projected area, length, width, perimeter (mm or cm), circularity (0 = elongated polygon, 1 = perfect circle), as well as the color through the values of RGB (Red, Green, Blue) or in CIELab (L= luminosity, 0 = black to 100 = white**;** a = red+/green- coordinates; b = yellow+/blue- coordinates). In addition, and the designed macro assigns a category or class for color and shape, an ordinal scale of size, and it can record the time of processing (useful tool for comparing with other methods).

The data obtained from variables is displayed in a customized table and automatically saved in the work directory as a comma-separated values file (csv) with the prefix "Results Count" plus the name of the processed image and the initials of the color space used. It also generates a table called "Summary Count", which shows the total number of grains recorded, the mean value of each variable, and the time consumed by each processed image. Both files can be edited in any electronic spreadsheet (MS Excel®, Gnumeric, OpenCalc OpenOffice and Calc LibreOffice). MacGran-IJ can also generate and save an image with colored grains identified by length and labeled with a number based on their position in the image to identify possible segmentation errors in images with grains in contact with each other.

The accuracy, precision and efficiency of the MacGran-IJ software were evaluated in comparison to the manual measurement of grain width and length, and they were also compared with the data for grain area, perimeter, length, width and circularity obtained using the SmartGrain software (available for Windows® in http://phenotyping.image.coocan.jp/smartgrain/SmartGrain.zip). SmartGrain does not record grain color, and it requires images with separated grains and user intervention to establish the scale and to contrast between the background and the grain. The results are saved as csv file (Tanabata *et al*., 2012).

The GrainScan software was also used to measure the following grain characteristics: area, perimeter, length, width, and color (only in the RGB space). GrainScan can separate grains in contact, yet it excludes circularity and it requires the introduction of the image resolution, the threshold for segmentation (the automated threshold with a sensitivity of 0.5 was used), as well as the definition of the minimum width and length of the grain in order to exclude undesired objects. The results are saved automatically in the Results directory in a file with the same name as the image and in csv format. Likewise, it generates images with the identified grains to visualize the accuracy of the segmentation. The software is only available for Windows® at https://data.csiro.au/dap/landingpage?execution=e3s2 (Whan *et al*., 2014).

Grain measurements were taken in two types of wheat, durum and bread, each represented by five genotypes. From each variety, a random sample of 50 whole and healthy grains was taken. Length (mm), ventral width (VWG, mm), and lateral width (LWG, mm) were manually recorded, all measured with a digital AutoTEC™ caliper (accuracy = 0.01 mm, range 0–150 mm). The grains were digitized using an Epson Stylus® CX4700 desktop scanner. Due to the complex shape, each grain was measured twice, once on the ventral side and once on the lateral side. Grains were placed separately on the crystal board of scanner to ensure the area of interest was obtained. Black foamy paper (ethylvinylacetate) was placed on bottom of the scanner cover to eliminate shadows, enhance contrast between background and grains, and facilitate image segmentation.

The image file format used was an RGB color jpeg, with 24 bits per pixel, a resolution of 300 dpi (dots per inch), dimensions of 2550 (width) x 3508 (height) pixels, and a file size of approximately 860 kB. The time it took to place 50 grains digitalize them was approximately 38.02 s when using a 64-bit Windows 8® operating system on a computer with an Intel Pentium® G630 processor running at 2.7 GHz and 3 GB of RAM. The morphological characteristics measured of each grain for the ventral (V) and lateral parts (L) were: projected area (SLG, SVG, mm^2), length (LLG, VLG, mm), width (LWG, VWG, mm), and perimeter (mm). The grain color was measured in the RGB color space using GrainScan and MacGran-IJ software; the latter was also used to obtain the color in the CIELab space. The total time (s) was recorded with a stopwatch for the caliper method and the digital image analysis (DIA) methods, where the digitization time was added.

The data were analyzed using R software (R Core Team, 2021) after verifying normality and homogeneity of variances. The descriptive statistical parameters of lateral and ventral grain lengths and grain widths obtained with a caliper and DIA software were compared. The accuracy was evaluated within each method by the coefficient of variation (CV) in six measurements of the same sample and between methods using Fisher's F test for the equality of variances. For evaluating accuracy, the mean relative error (MRE) was considered, along with the root-mean-square deviation (RMSD). Pearson's simple correlation (r) was also used, along with the coefficient of determination (R^2) for comparison between methods. The t test for independent samples was used to compare the wheat species (durum *vs.* bread wheat) in grain size, length, width, perimeter and circularity, as well as for the comparison between the lateral and ventral views of the grain.

The effect of the method on measuring size, length, width, perimeter, circularity, and color were analyzed separately for each type of grain using a parametric ANOVA. A nested design was used, in which the methods were the factor with the highest hierarchy and with a fixed effect, whereas genotypes were considered to have random effects and nested in each method. Tukey's test was used to compare means only when there were differences between methods ($p \leq 0.05$). The Kruskal-Wallis ANOVA was used for the time (s) recorded by the methods, and Dunn's test was used for the multiple comparisons of means between intervals. The time required to obtain the color in the CIELab and RGB spaces was compared using Wilcoxon's test for paired samples.

RESULTS AND DISCUSSION

The lateral length of the grain (LLG) was equal between methods ($p \geq 0.66$). With a caliper, the LLG in durum wheat was 7.03 mm while it was 6.06 mm in bread wheat grains; when using the MacGran-IJ method, durum wheat measured 7.28 mm and bread wheat 6.39 mm. In both wheat species, the GrainScan software recorded the highest LLG while the lowest was obtained with SmartGrain (Table 1). This result corresponds with observations by Whan *et al*. (2014), who reported a greater size, length, and width of wheat grains when measuring them with GrainScan compared to SmartGrain; this result may be attributed to the latter software running a morphological erosion process (*cvErode*) to remove the elongated ends, that is, pedicels and awns attached to the grain, thus resulting in lesser perimeter and area values, as previously mentioned by Tanabata *et al*. (2012).

The ventral length of the grain (VLG) in durum wheat obtained by using MacGran-IJ varied from 5.50 to 8.61 mm, and in bread wheat from 5.08 to 7.63 mm, with significant difference between both species ($p \leq 0.01$). These data coincide with the results for

Table 1. Lateral and ventral length of durum and bread wheat grains (n = 250) measured with a caliper by using three DIA methods.

Grain type	Method	Lateral length(mm)					Ventral length (mm)				
		Mean	Min	Max	SD	CV	Mean	Min	Max	SD	CV
Durum	Caliper	7.03	5.34	8.18	0.50	7.14	7.03	5.34	8.18	0.50	7.14
	MacGran-IJ	7.25	5.48	8.81	0.53	7.25	7.19	5.50	8.61	0.52	7.22
	SmartGrain	7.06	5.04	8.56	0.55	7.83	6.91	5.30	8.20	0.53	7.71
	GrainScan	7.41	5.54	8.82	0.53	7.15	7.28	5.52	8.60	0.51	7.03
Bread	Caliper	6.06	4.86	7.21	0.48	7.93	6.06	4.86	7.21	0.48	7.93
	MacGran-IJ	6.39	5.19	7.75	0.51	8.02	6.45	5.08	7.63	0.51	7.98
	SmartGrain	6.19	4.81	7.57	0.53	8.59	6.21	4.87	7.39	0.51	8.25
	GrainScan	6.49	5.16	7.81	0.51	7.91	6.61	5.14	7.85	0.53	7.96

Min: minimum; Max: maximum; SD: standard deviation; CV: coefficient of variation (%).

bread wheat reported by Goriewa-Duba *et al*. (2018). The VLG of durum wheat was statistically equal between methods ($p \geq 0.19$), with a low variation in each (CV ≤ 0.63 %), indicating its accuracy. The three DIA methods showed a slightly lower VLG compared to LLG (Table 1), as well as differences between the ventral and lateral lengths only when measured with SmartGrain ($p \leq 0.01$).

The LLG displayed a positive linear association between methods; in durum wheats, the coefficient of determination (R^2) between the length manually measured with a caliper and the MacGran-IJ method was 0.95 ($p \leq 0.01$). Similarly, Whan *et al*. (2014) reported an R^2 of 0.93 between grain length measured with SmartGrain and GrainScan. The lowest R^2 occurred between the GrainScan and manual caliper methods for both types of wheat (Table 2). The LLG of durum wheat measured with a caliper had a relative mean error (RME) of 3.10 % and a Standard Deviation (SD) of 1.61 % in regard to the value measured with MacGran-IJ, whereas the RME was 0.44 ± 2.20 % (SD) in relation to SmartGrain, and 5.44 ± 2.26 % (SD) when compared to GrainScan.

Table 2. Coefficient of determination (R^2), MRE, and RMSD between the manual method with a caliper and the DIA-based methods for measuring lateral and ventral lengths in 250 grains of durum and bread wheats.

Grain length	Grain type	Caliper *vs.*								
		MacGran-IJ			SmartGrain			GrainScan		
		R^2	MRE	RECM	R^2	MRE	RECM	R^2	MRE	RECM
Lateral	Durum	0.95	3.10	0.25	0.93	0.44	0.15	0.91	5.44	0.41
	Bread	0.91	5.53	0.37	0.89	2.22	0.19	0.88	7.19	0.47
Ventral	Durum	0.96	2.20	0.19	0.90	-1.80	0.20	0.90	3.53	0.29
	Bread	0.92	6.45	0.41	0.94	2.45	0.20	0.92	9.13	0.57

MRE: Mean relative error (%); RMSD: Root-mean-square deviation.

The association level between the LLG obtained with DIA and manual measurement was lower in bread wheat (Table 2). The grain length measured with DIA displayed a high adjustment in durum wheat ($R^2 \geq 0.90$), although accuracy was lower in bread wheat since RMSD and MRE increased between the measurement and the DIA-based methods (Table 2). This situation can be attributed to the irregularity in the body of the bread wheat grain, which induces slight inclinations when holding it with the caliper so then affecting its measurement.

The VLG obtained with a caliper presented a narrow positive linear association with MacGran-IJ, SmartGrain and GrainScan, with an R^2 of 0.94, 0.92 and 0.91 ($p \leq 0.01$) respectively, which are higher coefficients than those obtained by Zhu *et al*. (2021) for the length of rice grain, whom for manual measurement had an R^2 of 0.86 in regard to the values obtained with the SeedExtractor software, 0.70 with GrainScan, and 0.85 with SmartGrain. The R^2 of this study on wheat exceeded the 0.74 obtained by Miller *et al*. (2016) in maize grains measured with a caliper and with DIA, but was lower than the 0.97 R^2 estimated by LeMasurier *et al*. (2014) for lentil grains. Sharma *et al*. (2021) reported an R^2 of 0.81 in damaged wheat grain and of 0.95 in healthy whole wheat. The grain length, both lateral and ventral, showed differences between durum and bread wheats ($p \leq 0.01$).

The lateral width of the grain (LWG) measured with a caliper was lower value than that registered with DIA ($p \leq 0.01$). In durum wheat it was 2.89 mm (DSH = 0.22), and in bread wheat 2.87 mm (DSH = 0.33). On the other hand, the ventral width of the grain (VWG) was similar between the methods ($p = 0.40$). The coefficient of variation (CV) in the LWG in durum wheat was lower than 9.57 % and lower than 10.75 % in bread wheat, which decreased in VWG (Table 3). The lowest CV displayed by length (Table 1) in grain width agrees with other studies on wheat (Abdipour *et al*., 2016; Jamil *et al*., 2017; Alemu *et al*., 2020; Zhu *et al*., 2021). This response may be attributed to the fact that wheat grain width, as opposed to length, is more affected by adverse weather conditions (Jamil *et al*., 2017).

Table 3. Descriptive statistics of lateral and ventral grain width in durum and bread wheats (n = 250) obtained using a caliper and with three DIA-based methods.

Grain type	Method	Lateral width (mm)				CV	Ventral width (mm)				CV
		Mean	Min	Max	SD		Mean	Min	Max	SD	
Durum	Caliper	2.89	2.03	3.57	0.28	9.57	3.09	2.08	3.65	0.26	8.54
	MacGran-IJ	3.33	2.38	4.07	0.30	9.15	3.17	2.31	3.76	0.25	7.90
	SmartGrain	3.19	2.24	3.96	0.29	9.23	3.14	2.25	3.66	0.25	7.92
	GrainScan	3.27	2.30	3.96	0.30	9.04	3.14	2.00	3.80	0.27	8.53
Bread	Caliper	2.87	2.22	3.35	0.24	8.38	3.25	2.43	3.91	0.26	8.15
	MacGran-IJ	3.34	2.46	4.15	0.34	10.25	3.33	2.37	4.04	0.29	8.67
	SmartGrain	3.29	2.44	4.09	0.35	10.75	3.30	2.44	3.98	0.29	8.79
	GrainScan	3.35	2.52	4.10	0.34	10.13	3.39	2.47	4.07	0.29	8.46

Min: minimum; Max: maximum; SD: standard deviation; CV: coefficient of variation (%).

The manual method using a caliper reduces dimensions of the wheat grain, which according to Sun *et al*. (2007) is due to the pressure exerted on the grain when holding it with the caliper, which compresses it and reduces the obtained values. Manual measuring of the grains is also indicated by Hu and Zhang (2021) as prone to errors and inconsistencies; additionally, these authors claim that the accuracy of this manual method may be insufficient to become a standard recommended technique.

The LWG for durum wheat measured with a caliper showed a positive linear association with the measurements obtained using the MacGran-IJ, SmartGrain, and GrainScan software ($p \leq 0.01$). This relation decreased in bread wheat, which had a lower adjustment with $R^2 \leq 0.55$ (Table 4). The ventral width measured using a caliper and DIA outperformed the LWG in terms of accuracy, presenting a lower coefficient of variation and values ranging from 0.21 to 0.38 % in durum wheat, and 0.44 to 0.63 %

Table 4. Coefficient of determination (R^2), MRE, and RMSD between the caliper and DIA-based methods to measure the lateral and ventral lengths of 250 durum and bread wheat grains.

Grain width	Grain type	Caliper *vs.*								
		MacGran-IJ			SmartGrain			GrainScan		
		R^2	MRE	RMSD	R^2	MRE	RMSD	R^2	MRE	RMSD
Lateral	Durum	0.73	12.95	0.40	0.71	10.70	0.34	0.69	13.38	0.42
	Bread	0.55	16.50	0.52	0.50	14.59	0.49	0.51	16.91	0.54
Ventral	Durum	0.94	2.95	0.11	0.92	1.90	0.09	0.87	1.94	0.11
	Bread	0.91	2.48	0.12	0.90	1.57	0.11	0.92	4.45	0.17

MRE: mean relative error (%); RMSD: root-mean-square deviation.

in bread wheat. Accuracy also improved in VWG, which was reflected by lower MRE and RMSD values.

The highest MRE in the LWG corresponds to the sharpened shape of the dorsal part of the wheat grain, which allows for two different lateral views. In addition, it is difficult to locate the exact point to measure the grain width with a caliper, and therefore it differs with the DIA. This also occurred with the rice grain, where Zhu *et al*. (2021) found low accuracy between the grain obtained with a caliper and with DIA, but they did not specify the reason. Our findings claim that this can be explained by the lower repetitiveness for recording the same LWG with a caliper, as compared to the VWG. This is because the ventral part is unique and easy to identify, which always guarantees a measurement on the same side of the grain; additionally, the ventral part allows a higher rate of coincidences between records.

The VWG manually measured displayed an $R^2 \geq 0.90$ for bread wheat, and a lower value of 0.87 for durum wheat ($p \leq 0.01$), indicating a narrow relation that coincides with studies showing similar comparisons. Hu and Zhang (2021) and Sharma *et al*. (2021) reported an R^2 of 0.92 between the ventral width measured with a caliper and DIA in whole and healthy wheat grains. The VWG obtained with a caliper was greater than the LWG, with no differences between them when measured with SmartGrain ($p \geq 0.28$). Between methods, there was strong positive linear correlations of 0.70, 0.78, 0.78, and 0.79 ($p \leq 0.01$), corresponding to measurements obtained with a caliper, MacGran-IJ, SmartGrain, and GrainScan. The digitization of wheat grains placed on their ventral side and separated has been used (Goriewa-Duba *et al*., 2018; Ropelewska, 2019; Zhang and Ji, 2019), but it is an impractical method due to its time-consuming nature. In contrast, the grains in our proposed methodology can come into contact with each other, but they were only spaced to compare with manual measurements.

For grain length, the accuracy achieved by MacGran-IJ, GrainScan, and SmartGrain was similar ($F \geq 0.9 \leq 1.09$, $p \geq 0.05$), while the accuracy of MacGran-IJ compared to SmartGrain had an MRE = -2.89 ± 1.92 %, and with GrainScan the values were 1.93 ± 1.89 %. When comparing between the three software, grain width had an $R^2 \geq 0.94$ with an MRE of -1.83 ± 2.08 % between MacGran-IJ and SmartGrain, and -0.38 ± 2.52 % with GrainScan. The R^2 values in both variables coincide with those obtained by Whan *et al*. (2014) when comparing GrainScan and SmartGrain.

The differences in the accuracy and precision between these softwares correspond to each one's own algorithm; however, along with efficiency and repetitiveness, they are affected when the processing lacks automation, because it requires user intervention to manually establish the scale or to carry out the segmentation between the objects of interest and the image background. This occurs with SeedExtractor because there are differences between users in the dimensions measured in the same wheat grain (Zhu *et al*., 2021); this risk is also present in GrainScan and SmartGrain.

Both lateral and ventral grain size, as well as perimeter, were larger when measured with GrainScan (Table 5), although only the perimeter showed a difference between methods ($p \leq 0.01$). The grain circularity was higher in bread wheat (0.75) and also in the ventral part (0.76), with differences between both species and between views of the wheat grain ($p \leq 0.01$), but without differences between methods ($p \geq 0.50$). In durum wheat grain, the ventral size was smaller than the lateral size ($p \leq 0.01$), whereas in bread wheat, the ventral part projected a larger area ($p \leq 0.01$). Between species, the durum wheat grain was larger and had a lower circularity. Goriewa-Duba *et al*. (2018) also pointed out that the size of the durum wheat grain is 19.55 mm^2 with a circularity of 0.69, whereas in bread wheat the corresponding values were 15.24 mm^2 and 0.76 respectively.

Table 5. Size, perimeter, and circularity measured from the lateral and ventral parts using DIA on 250 durum and bread wheat grains.

Grain type	Method	Size (mm^2)		Perimeter (mm)		Circularity (0-1)	
		Lateral	Ventral	Lateral	Ventral	Lateral	Ventral
Durum	SmartGrain	17.61	17.04	17.79 a	17.37 a	0.70	0.71
	MacGran-IJ	18.25	17.66	18.06 a	17.66 a	0.70	0.71
	GrainScan	19.18	17.99	21.57 b	20.67 b	-	-
	CV	13.92	13.10	7.12	6.57	3.89	3.61
	HSD ($p \leq 0.05$)	NS	NS	0.99	0.97	NS	NS
Bread	SmartGrain	15.69	16.25	16.18 a	16.29 a	0.75	0.76
	MacGran-IJ	16.33	16.98	16.49 a	16.56 a	0.75	0.77
	GrainScan	17.07	17.64	20.03 b	19.68 b	-	-
	CV	14.21	13.09	7.55	6.77	4.29	2.88
	HSD ($p \leq 0.05$)	NS	NS	1.27	1.23	NS	NS

Different letters in each column indicate statistical differences (Tukey, $p \leq 0.05$); NS: not significant; CV: coefficient of variation; HSD: honest significant difference.

The correlation between grain size and length was 0.88 for durum wheat and 0.84 in bread wheat ($p \leq 0.01$), and the correlation between grain size and width was 0.92 and 0.89, respectively. These values exceed the r of 0.74 reported by Abdipour *et al*. (2016)

between bread wheat size and length, and a r of 0.68 between bread wheat width. The ventral size measured with GrainScan showed no significant differences between species, although the size and the perimeter differed for the type of grain, as well as between the lateral and ventral views of the grain ($p \leq 0.01$).

The bread wheat grain color is lighter, as shown by its greater intensity of red, green and blue. The red color taken from the ventral and lateral parts of the durum wheat grain differed between GrainScan and MacGran-IJ methods ($p \leq 0.05$). In bread wheat, the methods are different for red, green and blue on the lateral side of the grain ($p \leq 0.05$), but there were no differences on the ventral side (Table 6). The ventral and lateral grain color displayed a close positive linear correlation between GrainScan and MacGran-IJ (r ≥ 0.97, $p \leq 0.01$), which agrees with Whan *et al*. (2014) who reported a correlation of more than 0.99 between the wheat grain color obtained with GrainScan, the SeedCount® system and the Minolta CR-400® colorimeter. The strong association between the colorimeter and the DIA is due because both measure color based on light reflectance in the visible spectrum.

Table 6. Mean intensity of the red, green, and blue colors obtained using DIA from the lateral and ventral parts of 250 grains of durum and bread wheats.

Grain type	View	Method	Color Red (R)	Green (G)	Blue (B)
Durum	Lateral	GrainScan	143.08 a	114.57	97.30
		MacGran-IJ	136.41 b	109.56	93.50
		CV	3.03	3.87	4.76
		DSH ($p \leq 0.05$)	4.62	NS	NS
	Ventral	GrainScan	141.71 a	112.31	94.98
		MacGran-IJ	135.42 b	107.59	91.33
		CV	3.39	4.43	5.56
		DSH ($p \leq 0.05$)	5.42	NS	NS
Bread	Lateral	GrainScan	150.26 a	119.78 a	102.52 a
		MacGran-IJ	140.99 b	112.57 b	96.74 b
		CV	3.17	4.09	4.83
		DSH ($p \leq 0.05$)	4.43	5.00	2.15
	Ventral	GrainScan	147.81	115.65	98.01
		MacGran-IJ	140.30	110.29	93.89
		CV	2.99	3.72	4.30
		DSH ($p \leq 0.05$)	NS	NS	NS

Different letters in each column indicate statistical differences (Tukey, $p \leq 0.05$); NS: not significant; CV: coefficient of variation; HSD: honest significant difference.

Color was obtained in CIELab in approximately 11.57 s and and measured in the RGB space in approximately 7.25 s, which is approximately 37.34 % less time ($p \leq 0.01$). The DIA is still scarcely used, despite advances in computing, to measure the wheat grain color and has a low efficiency when implemented on images of a single grain (Ropelewska, 2019) or with a sample of grains but digitized with a distance between each (Goriewa-Duba *et al*., 2018; Bao *et al*., 2019; Zhang and Ji, 2019; Alemu *et al*., 2020; Sharma *et al*., 2021), and even less efficient when the process is semiautomatic. On the other hand, in GrainScan and MacGran-IJ, the grains can be in contact, and with the latter, the measurement is automated.

Measuring the length and width of 50 grains with a caliper from the lateral and ventral views took 1528.07 s, with a standard deviation (SD) of 92.42 s; SmartGrain took 101.84 ± 0.7 s, MacGran-IJ 45.4 ± 0.38 s, and GrainScan 54.11 ± 0.17 s (H = 36.59, $p \leq 0.01$). Whan *et al*. (2014) reported around 210 s with SeedCount® and less time (101 s) with SmartGrain and GrainScan software to analyze samples ranging from 382 to 985 grains, whereas Zhu *et al*. (2021) claim that measuring rice grains digitized with a scanner required 61.46 s with SmartGrain, 13.22 s with GrainScan and 2.14 s with SeedExtractor, not including the time required to digitize.

The time required to take measurements manually depends on the dimensions, and the grain shape, as the smaller the latter, the greater the difficulty in handling it, and the greater the irregularity, the greater the difficulty in measuring it. Miller *et al*. (2016) implemented, in Matlab®, a DIA-based methodology that was five times faster to measure the length of 100 maize grains and the dimensions of three ears in comparison to measurements taken using a caliper. MacGran-IJ was approximately 17 times faster than the manual length and width recording process of 50 grains on the lateral and ventral part; additionally, in the same amount of time, it recorded six variables related with dimensions and five related to color.

The time required for DIA depends on image resolution and size, software, the computer used, the number of obtained variables, as well as the complexity of the processing and the automation of analysis. The efficiency is also improved by the option to analyze a batch of files, which is available in GrainScan, SmartGrain and MacGran-IJ, however, only MacGran-IJ can correctly handle images with different resolutions. The efficiency of DIA is related with the number of grains digitized in an image, as well as the time required to digitize them (the most time-consuming part, since it is carried out with a scanner), and the time required by the DIA. When the total time is divided by the number of grains analyzed, the efficiency is greater as the number of grains increases.

CONCLUSIONS

MacGran-IJ is a simple and efficient method for accurately measuring the color and projected area, as well as the length and width of each wheat grain, by analyzing images captured with a desktop scanner. The hypothesis that MacGran-IJ efficiently and accurately measures grain morphological and color variables was confirmed. GrainScan, SmartGrain and MacGran-IJ software are all effective and accurate for measuring wheat grains, although efficiency is greater with GrainScan and MacGran-IJ. The advantage of the latter is the larger number of variables it provides and its simplicity, due to it being automated.

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