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Article

Leaf and grain morphology of *Glycine max* L. using digital images

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Abstract

Leaf and grain morphology of soybean (*Glycine max* L.) is necessary to identify varieties and explain their agronomic behavior, but it requires quantitative and easy-to-obtain measurements. This can be solved by digital image analysis (DIA); therefore, it was implemented to evaluate the leaf and grain morphology in the Cajeme, Guayparime S-10, and Harbar '88 varieties. The DIA was automated in ImageJ 1.51 t to measure size, length, width, circularity, and color in leaflets (cm) and grains (mm). Specific leaf area (SLA, cm^2 g^{-1}), total grain area (TA, mm^2), number of pods (NPP) and grains per plant (NGP), hectoliter weight (HEW, kg hl^{-1}) and 100 grains (WHG, g) were also measured. The central leaflet was elliptical in shape, larger in size, and had SLA ($p\leq$ 0.05), while the lateral ones were oval. Leaflet area (LA) was correlated ($p\leq$ 0.01) with length, width, and their product (r# 0.93). Cajeme showed different leaf color ($p\leq$ 0.01); Guayparime S-10 had higher LA, HEW, NPP and NGP but a grain that is smaller in size, length and width ($p\leq$ 0.01). WHG was associated ($p\leq$ 0.01) with TA (r_s= 0.89), size (r_s= 0.88), grain length and width ($r_s\leq$ 0.71), and leaf size (r_s= -0.5). Harbar '88 showed brighter grain, and Guayparime S-10 smaller grain ($p\leq$ 0.01). The circularity of the leaflet facilitates the objective classification of the shape. The DIA is useful for phenotyping; it allows the identification of differences in leaflets and grains of the Cajeme, Guayparime S-10, and Harbar '88 varieties.

Keywords:

Glycine max, color, ImageJ, shape, size.

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Introduction

The foliar and grain morphology of soybean (*Glycine max* L.) is important in plant breeding, physiology, and nutrition. Leaf area, shape, and weight are related to light distribution, photosynthetic efficiency (Krisnawati and Adie, 2017; Schwerz *et al*., 2019), drought tolerance, and yield (Jun *et al*., 2014). Leaf color indicates health and nutrition (Kumar *et al*., 2017). Grain size, shape, and color are associated with yield and quality and allow its classification and the study of its heritability (Hu *et al*., 2013), helpful in designing harvesting and processing equipment (Rehal *et al*., 2019).

The shape of the leaflets and grain is estimated qualitatively and quantified by the relationship between length and width (Liang *et al*., 2016; Krisnawati and Adie, 2017). Leaf color is associated with SPAD 502® readings (Sauceda *et al*., 2017a) and grain color with colorimeters (Rehal *et al*., 2019). The thickness, size, and variation of leaf color limit the readings of the SPAD 502® and force an increase in replications (Sauceda *et al*., 2017a); a similar situation occurs with colorimeters for grain (Yousif, 2014).

Leaf area is estimated with regression models based on central leaflet length (Richter *et al*., 2014) and measured with LI-COR 3000 (Schwerz *et al*., 2019). Quantitative measurement is slow and tedious, even with digital calipers (Richter *et al*., 2014, Liang *et al*., 2016). Leaf variability restricts the use of a general model to estimate area (Richter *et al*., 2014). The LI-COR 3000 measures only leaf area and has drawbacks that require repetition or prevent measurement (Sauceda *et al*., 2017a).

Visual recording of leaf and grain morphology is easy and fast but subjective, with low repeatability and prone to errors; therefore, UPOV suggests quantitative description through digital image analysis (DIA), which is little used (UPOV, 2013). DIA is an economical option as it is performed with a scanner and free programs (Jun *et al*., 2014), although it requires automation for greater efficiency, accuracy, and ease of adoption.

Therefore, DIA was used to evaluate the morphology of soybean leaflets and grains, with the hypothesis that the attributes obtained with DIA automated in ImageJ 1.51t will allow differences between varieties to be verified.

Materials and methods

The study was conducted at the Valle del Fuerte Experimental Field of INIFAP, Mexico, with clay-sandy soil. The Cajeme, Guayparime S-10, and Harbar ’88 soybean varieties were evaluated in deep, saline-free clay-sandy soil. The experimental design was randomized blocks with four replications. The plot consisted of four furrows with a separation of 80 cm and a length of five meters.

Fresh mature leaves were randomly collected during grain filling; the leaflets were classified according to their position (central, left, and right) on the leaf, seen by the adaxial side and with the central leaflet facing upwards. The digitization was done with an Epson CX3900 scanner; for the leaflets, a white background was used, and for grains, a black one. The format of the images was color jpeg, with a resolution of 300 pixels per inch (ppi).

Digital image analysis (DIA) was automated with ImageJ 1.51t (Sauceda *et al*., 2017a) to measure the color, size, length, width, and circularity of leaflets (cm) and grains (mm). Leaf color was measured in RGB, and grain color in Lab. The grain color was also measured with a HunterLab MiniScan colorimeter, model EZ Plus 4500-L (Hunter Associates Laboratory Inc., Reston, Virginia, USA).

The leaflet shape was classified as lanceolate, elongated, elliptical, and oval based on the leaflet length-to-width ratio (LLWR) and the circularity of the lateral and central leaflets represented in the distinctness, uniformity, and stability (DUS) test guides for soybean varieties, provided by UPOV (UPOV, 1998; 2017).

The product between the length and width (LWL) of the leaflets: central, right, left, and the average of the three (CRL) together with their leaf area were used to generate linear regression models with

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forced zero intercept due to its greater biological explanation (Richter *et al*., 2014). The SLA per leaflet ($cm^2\ g^{-1}$) was calculated in 57 samples per variety; the biomass (g) was obtained after 72 h of drying at 65 °C.

The length, width, and thickness of 60 grains per variety were measured with a Truper™ digital vernier (accuracy= 0.01 mm) to obtain, by analogy to a sphere, the mean geometric diameter [D_g= $(\text{length x width x thickness})^{1/3}$] and estimate the total area of the grain (TA, mm^2) with the equation: $TA= \pi D_g^{\ 2}$ (Rehal *et al*., 2019). It was also done to compare the dimensions and the time(s) required to measure them with vernier and DIA.

The number of pods and grains in ten plants was obtained from each variety; the size of the grain of the upper and lower half of the plant was measured in these. Grain size was related to the weight of 100 grains (WHG, g) and the weight of the grain per plant (WGP, g). The weight was obtained with an Ohaus SC2020 Scout® II scale.

Hectoliter weight (HEW, kg hl^{-1}) and grain moisture (%) were obtained with a Dickey-John grain analyzer, model GAC2000. Data analysis was carried out using the Past 3.18 program (Hammer *et al*., 2001), and normality (Shapiro-Wilks), homoscedasticity (Levene), and independence of the residuals (Durbin-Watson) were verified. The data on the leaflet area was transformed into a square root.

The Anova and mean comparison (Tukey, $p\leq 0.01$) was performed for the area, length, and width of the leaflet, as well as for the size and length of the grain. The t-test for independent samples was used to compare the grain width of the upper and lower middle part of the plant. The Pearson correlation (r) was calculated between the leaflet area with its length, width, and the LWL; the models generated to estimate the leaf area were evaluated using the coefficient of determination (R^2), the mean relative error (MRE), and the root mean square error (RMSE).

The Pearson correlation (r) was calculated between grain size, TA, length, product (LWG), and grain length-to-width ratio (GLWR), number of pods and grains per plant, also between the average area of the three leaflets and the WHG. The lack of normality for the circularity and color of both organs in the LLWR, grain width, and WHG motivated us to use the Kruskal-Wallis Anova and Dunn's test for the multiple comparison between the mean ranges by pairs of samples.

The Mann-Whitney test was used to assess the size, length, circularity, and color of the grain of the upper and lower middle part of the plant. Wilcoxon's test was used to compare grain color measured with a colorimeter and DIA. The Spearman correlation (r_s) was made between the TA, size, length, width, and the WHG and between the LLWR and the circularity of the leaf.

Results and discussion

The size of the central leaflet presented, on average, 31.17 cm^2 ±7.61 cm^2 (standard deviation, SD); the lateral ones had smaller size (LSD= 2.48, $p\leq 0.01$) and slightly greater variation (4.5%). The leaf area per leaf of the Harbar '88 variety was 70.62 cm^2, Cajeme had 88.99 cm^2 and Guayparime S-10 showed 94.35 cm^2 (LSD= 12.52, $p\leq 0.01$), values similar to the maximum of 85 cm^2 reported by Jun *et al*. (2014). Guayparime S-10 had 32.02 cm^2 in leaflet size and differed from Harbar '88 (LSD= 2.3, $p\leq 0.01$).

The central leaflet was larger, with a size ranging from the minimum (23.23 cm^2) to the maximum (39.82 cm^2) reported by Sayama *et al*. (2017) in 12 soybean genotypes. Leaflet size presented a high coefficient of variation (Table 1), also reported by other studies (Park *et al*., 2013; Khan *et al*., 2018), partly attributable to the fact that the dimensions vary according to their location on the plant (Schwerz *et al*., 2019), an important aspect when estimating leaf area.

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Table 1. Dimensions of the central, right, and lateral leaflet in three soybean varieties during the AW 2017-2018 cycle in Guasave, Sinaloa, Mexico.

Variety	Central leaflet			Right leaflet			Left leaflet		
	Area (cm^2)	L (cm)	W (cm)	Area (cm^2)	L (cm)	W (cm)	Area (cm^2)	L (cm)	W (cm)
Cajeme	32.98a	8.98a	5.23a	28.95a	7.6a	5.02a	28.59a	7.63a	4.97a
Guayparime S-10	33.89a	8.99a	5.31a	30.83a	7.83a	5.1a	31.33a	7.88a	5.16a
Harbar '88	26.65b	8.08b	4.71b	23b	6.88b	4.42b	22.31b	6.76b	4.36b
Mean	31.17	8.68	5.08	27.59	7.44	4.85	27.41	7.42	4.83
CV	22.23	11.61	11.66	26.42	13.14	13.91	25.74	12.63	14.05
LSD (Tukey $_{0.01}$)	3.79	0.55	0.32	3.99	0.53	0.37	3.86	0.51	0.37

n= 57; L= length; W= width; CV= coefficient of variation; LSD= least significant difference. Different letters in each column indicate significant differences.

The environment influences the dimensions of the leaflet (Jun *et al*., 2014), but the genotype *per se* also does so, as occurred in Harbar '88, as it showed leaflets of smaller dimension (Table 1), an aspect to be considered to define sowing density, since genotypes with small leaflets increase yield with higher population density, due to better light distribution (Krisnawati and Adie, 2017).

Guayparime S-10 presented greater leaflet circularity (H= 10.92, $p \leq 0.01$), with a mean range of 287.15 and a least significant difference of 46.81 (Dunn, $p \leq 0.01$). The ratio between leaflet length and width (LLWR) was similar between varieties and was correlated with circularity (r_s= -0.77, $p \leq 0.01$). The circularity of the central leaflet was lower (mean range= 138.54) than the lateral leaflets (H= 165.6, $p \leq 0.01$), where the LLWR was lower (H= 201.14, $p \leq 0.01$), the left leaflet had a range of 190.37 and the right leaflet 192.37 (Dunn= 47.05, $p \leq 0.01$).

The circularity of the leaflet presented a CV between 3.7 and 5.89%; the low variation is because the shape is little affected by the environment (Chen and Nelson, 2004). The circularity of leaflets in UPOV guides from 1998 and 2017 was 0.49-0.58 lanceolate, 0.59-0.68 elongated, 0.69-0.78 elliptical, and 0.79-0.88 oval. In all three varieties, the shape of the central leaflet was elliptical and it was oval on the lateral leaflets; however, the three leaflets are oval according to Chen and Nelson (2004), who group the elliptical shape with the oval shape and suggest a length-to-width ratio less than or equal to 2 for oval and 2.1 to 3 for elongated, between 3.1 and 4 for lanceolate, from 4.1 to 5 for linear, and greater than 5 for ultra-linear.

Using leaflet circularity to classify its shape is feasible since leaves with similar circularity values have a similar shape (Krieger, 2014). The classification by Chen and Nelson (2004) and the one obtained in this study made it possible to automate the DIA to classify the shape of soybean leaflets, to facilitate its obtaining by inexperienced personnel and to promote repeatability. The shape of the leaflet, except for the elliptical, makes it difficult to establish, with Vernier, the width to estimate the leaf size and shape, a problem solved with the DIA because it systematically defines the minor and major axis.

The leaflet area showed a correlation ($p \leq 0.01$) with its length (r= 0.93), width (r= 0.96), and LWL (r= 0.99); the greatest association between leaf size and LWL coincides with Richter *et al*. (2014), who point out greater precision and accuracy when estimating leaf area of the central leaflet by LWL. Estimating leaf area based on the central leaflet is prone to error as it differs in size and shape from the lateral ones, a risk in compound leaves noticed by Keramatlou *et al*. (2015). The generation and validation of models to estimate leaf area can be facilitated with DIA, and LI-COR 3000 can be omitted.

The leaflet area measured and estimated with a model based on the LWL of the central leaflet presented an R^2 of 0.99 ($p \leq 0.01$), with a higher mean relative error (MRE) of 4.73% ±4.18% (SD)

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and lower accuracy (RMSE=1.87 cm^2). The model with average LWL of the three leaflets (CRL) reduced precision (R^2= 0.97) but increased accuracy (Table 2). The RMSE of the models was low (≤1.87 cm^2). The precision was similar to that obtained by Richter *et al*. (2014) when estimating the area with the LWL of the central leaflet (R^2≥ 0.97), but they indicated lower accuracy (RMSE≥ 6.48 cm^2). The lower accuracy when using LWL of the central leaflet in this study is because it differs in shape and size from the lateral leaflets.

Table 2. Precision and accuracy of empirical models to estimate leaf area with the product of leaflet length and width (LWL), and the average of the three leaflets (CRL).

Leaflet	Model	R^2	Mean relative error (%)				RMSE (cm^2)
			Minimum	Maximum	Mean	SD	
Central	LA = 0.6961*LWL	0.99	-13.54	6.27	-4.73	4.18	1.87
Right	LA = 0.7512*LWL	0.99	-6.69	14.68	2.81	4.51	1.66
Left	LA = 0.7502*LWL	0.99	-6.82	14.52	2.67	4.5	1.64
CRL	LA = 0.7277*LWL	0.97	-9.61	11.09	-0.41	4.37	1.35

n= 513; LA= leaf area; SD= standard deviation; RMSE= root mean square error.

The leaflet area estimated by the average LWL of the three leaflets (CRL) showed lower MRE and RMSE (Table 2); the improvement in accuracy was due to the inclusion of the lateral leaflets, which are similar in shape and size. This is also evident when using specific models for the right and left leaflets, as the MRE decreased and the accuracy between the estimated leaflet area and the leaflet area measured with DIA increased (Table 2). Although Richter *et al*. (2014) propose using the central leaflet for practicality and time-saving, measuring a lateral leaflet is just as easy and offers greater accuracy. Nonetheless, the results of this research indicate that it is better to estimate the leaf area based on the LWL of the three leaflets.

The specific leaf area (SLA) was similar between varieties but varied according to the position of the leaflet on the leaf (LSD= 14.98, *p*# 0.05) and, according to Schwerz *et al*. (2019), it also varies with leaf position on the plant. The central leaflet had the lowest SLA, with 172.08 cm^2 g^{-1} (SD= 5.49); the left one had 182.54 cm^2 g^{-1} (SD= 5.32) and the right one, 190.85 cm^2 g^{-1} (SD= 10.7). The difference in SLA of the lateral leaflets is relevant due to its positive association with SPAD 502® readings (Sauceda *et al*., 2017a). The higher SLA of the lateral leaflets suggests a thinner leaf blade as a possible adaptation to the environment since the SLA increases due to agronomic practices such as sowing soybeans intercropped with corn (Liu *et al*., 2017) by modifying radiation.

The Cajeme variety showed a different leaf color, with less intensity in red (R), green (G), and blue (B) (Table 3); these low values indicate a more intense green and correlate with higher chlorophyll content (Kumar *et al*., 2017) and with SPAD 502® readings (Sauceda *et al*., 2017a). The association of color with SPAD 502® readings is negative and it is due to lower light reflection since greater absorption reduces the transmission and reflectance of electromagnetic waves (Jacquemoud and Ustin, 2008).

Table 3. Leaf color obtained by DIA in leaflets of three soybean varieties during the AW 2017-2018 cycle in Guasave, Sinaloa, Mexico.

Variety	Red (R)			Green (G)			Blue (B)		
	Ranges		Means	Ranges		Means	Ranges		Means
Cajeme	186.52	b	58.26	203.89	b	64.18	177.05	b	50.62

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Variety	Red (R)			Green (G)			Blue (B)		
	Ranges		Means	Ranges		Means	Ranges		Means
Guayparime S-10	289.39	a	61.43	294.98	a	66.43	295.28	a	52.8
Harbar '88	295.09	a	61.15	272.13	a	65.71	298.68	a	52.71
H (p≤ 0.01)	58.12			34.96			74.67		

H= Kruskal-Wallis statistic; minimum difference between ranges (Dunn= 47.05); different letters by columns indicate significant differences.

RGB values are helpful in diagnosing physiological stress due to nitrogen or water (Kumar *et al*., 2017), but they can also reflect genotypic differences, as in the Cajeme variety, the leaf coloration of which was different despite homogeneous conditions. The DIA allowed measuring the color and leaf dimensions in 2.72 s, automation favored time due to less user intervention (Sayama *et al*., 2017). Digitizing four leaves per image (12 leaflets) took 35 s.

The Harbar '88 variety had the largest grain size (29.9 mm^2), while Guayparime S-10 had the smallest grain (Table 4). Grain size showed a significant negative association (p≤ 0.01) with the number of pods (r= -0.7) and grains per plant (r= -0.72). In addition, the number of grains per plant was related to the individual weight of the grain (r_s= -0.58, p≤ 0.01); this inverse relationship is also pointed out by Sayama *et al*. (2017), even between grains of the same pod.

Table 4. Number of pods and grains, weight of grain per plant (WGP, n= 10), grain dimensions (n= 1 000), and hectoliter weight (HEW, n= 3) in three soybean varieties during the AW 2018-2019 cycle in Guasave, Sinaloa, Mexico.

Variety	No. per plant		Length (mm)	Size (mm^2)		LWG (mm^2)	HEW (kg hl^{-1})	WGP (g)
	Pods	Grains		Area	Total			
Cajeme	22.9 ab	56.2 ab	6.5 b	28.6 b	94.2 b	37.4 b	71.7 b	6.7 b
Guayparime S-10	30.1 a	74.2 a	6.5 b	27.9 c	91.7 c	36.5 c	74.4 c	7.4 b
Harbar '88	17.1 b	38.1 b	6.7 a	29.9 a	98.6 a	39.2 a	70.7 a	4.6 a
Mean	23.4	56.2	6.5	28.8	94.8	37.7	72.3	6.3
CV	24.3	25.3	7.1	12.9	12.9	13	0.3	25
LSD (Tukey $_{0.01}$)	8.1	20.2	0.1	0.5	1.6	0.64	0.6	1.7

Different letters in each column indicate significant differences. CV= coefficient of variation; LSD= least significant difference; LWG= length by width of the grain.

The higher grain production per plant of the Guayparime S-10 variety can be related to its high productive potential (Rodríguez *et al*., 2017) since soybean yield is favored more by the number of grains per plant than by their size or individual weight (Hu *et al*., 2013; Schwerz *et al*., 2019), as reflected in the higher WGP (Table 4). The higher hectoliter weight (HEW) confirmed the smaller grain size of the Guayparime S-10 variety (Table 4); since the weight-volume ratio is affected by the size and shape of the grain, the lower compaction of large grains is due to more empty spaces between them; De la O *et al*. (2012) indicate that small wheat grains show greater HEW than large and elongated ones.

The upper middle part of the plant presented grain of greater size, length, width, circularity, and color compared to the lower middle portion (p≤ 0.01); the lower weight of the grain in the lower stratum corresponds to the lower photosynthetic activity of the leaves located in the lower part (Schwerz *et al*., 2019), due to self-shading and greater leaf senescence in reproductive stages, such as grain filling. The decrease in the photosynthetic rate translates into less photosynthate disposition for the grain. This highlighted the relevance of the light distribution in the canopy on the uniformity of the grain.

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The grain length obtained by the DIA was different between the genotypes and with a low coefficient of variation (Table 4), which is consistent with the reduced variability of this characteristic reported by Hu *et al*. (2013), also evident in the results (# 8.25%) of Rehal *et al*. (2019). The grain width of Guayparime S-10 was 5.63 mm, while Cajeme showed 5.73 mm, and Harbar '88 presented 5.87 mm (H= 142.44, $p\leq 0.01$). The LWG was higher in the Harbar '88 variety (Table 4). The moisture of the grain was 7.32%, with no difference between the varieties.

The grain length obtained with vernier and by DIA showed a high correlation (r= 0.92, $p\leq 0.01$), but the association between both methods to measure width decreased (r= 0.77, $p\leq 0.01$). This decrease is associated with the size and ellipsoid shape of soybean grains, which make it difficult to measure the width accurately, even with a digital caliper, due to the variability in the location of the measurement, which affects repeatability.

The TA showed association with the area of the grain (r= 0.87, $p\leq 0.01$), with LWG (r= 0.84, $p\leq 0.01$), and also with its length and width measured with DIA (r= 0.72, $p\leq 0.01$). The DIA needed 55.97 s to obtain the dimensions and color in samples of more than 300 grains; in contrast, measuring with vernier, only the length, width, and thickness of 60 grains took 3 718.2 s. The efficiency and repeatability of measuring leaf and grain morphology with DIA are favored by automation, whose implementation in free-to-use programs represents an opportunity to encourage the adoption of the methodology.

The circular shape of the grain was higher in Cajeme and Harbar than in Guayparime S-10 (H= 134.71, $p\leq 0.01$). The weight of 100 grains (WHG) ranged from 9.6 to 12.5 g, with a CV= 8.61%, and had a strong correlation ($p\leq 0.01$) with size (r_s= 0.88), length (r_s= 0.81), width (r_s= 0.89), LWG (r_s= 0.88), GLWR (r_s= -0.71), and TA of the grain (r_s= 0.89). These relationships are consistent with other soybean studies (Hu *et al*., 2013; Liang *et al*., 2016).

The smaller grain width reduces weight but increases GLWR, which is reflected in the negative relationship between GLWR and WHG. The mean WHG was 12 g in Harbar '88, 11.89 g in Cajeme, and 10.1 g in Guayparime S-10 (H= 20.24, *p*# 0.01). Leaf area showed a negative correlation with WHG (r_s= -0.5, $p\leq 0.01$), a similar association (r= -0.41, $p\leq 0.05$) to that indicated by Khan *et al*.(2018), but which differs from the positive correlation pointed out by Park *et al*. (2013) between the soybean WHG and the area of the third trifoliate leaf. The discrepancies in the relationship between both variables are due to the influence of the shape, angle, and arrangement of the leaves in the canopy of the soybean plant.

The grain color differed between the varieties; Harbar '88 presented a more yellow and lighter grain, evident in the greater lightness (Table 5). Positive values at coordinates *a* (red/green) rule out the presence of immature or green grains and coordinates *b* (yellow/blue) confirm the yellow hue (Rehal *et al*., 2019).

Table 5. Color (Lab) of grain obtained by DIA in three soybean varieties during the AW 2017-2018 cycle in Guasave, Sinaloa, Mexico.

Variety	Lightness			a			b		
	Ranges		Means	Ranges		Means	Ranges		Means
Cajeme	1 087.8	a	56.46	1 158.09	a	7.02	1 380.08	a	17.92
Guayparime S-10	1 619.23	b	57.81	1 379.37	b	7.24	1 398.02	a	18.07
Harbar '88	1 794.48	c	58.12	1 964.04	c	7.87	1 723.4	b	19.02
H ($p\leq 0.01$)	361			462.23			99.55		

n= 1 000; different letters in each column indicate significant differences; minimum difference between ranges (Dunn= 47.05).

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The yellow color of the grain was uniform in the three varieties: Guayparime S-10 showed a variation of 16.44%, Cajeme of 12.95%, and Harbar of 11.64%, indicating the absence of damaged grains. The measurement of soybean grains by the DIA presented precision, accuracy and efficiency; nevertheless, its use in phenotyping is still limited (UPOV, 2013).

Color differs between measurements with the DIA and the HunterLab MiniScan colorimeter ($p\leq$ 0.01). This discrepancy could be attributed to the restricted measurement area of the colorimeter, with a diameter of 25 mm, which forces one to increase the replications to achieve greater representativeness and reliability in heterogeneous samples. The replications per grain sample are indefinite; in this study, there were ten, but Yousif (2014) suggests up to 60 measurements to obtain an average value for soybean grains.

The increase in observations reduces efficiency due to the greater time invested; on the other hand, with the DIA, measuring the color and dimensions of each grain is fast and, despite this, it is little used; however, when the grains are digitized with manual separation, the efficiency decreases (Sauceda *et al*., 2017b); in the present work, the obtaining of the morphology and color was automated and with grains in contact; in this way, the time spent digitizing and analyzing samples decreases.

Conclusions

The differences in the Cajeme, Guayparime S-10, and Harbar ’88 soybean varieties are confirmed by characteristics extracted from digital images, such as size, circularity, and color of leaflets and grains. The shape of the soybean leaflet and grain makes it difficult to measure their width consistently with the vernier, while digital image analysis ensures repeatability as it is a systematic process.

The average product of length by width of the three leaflets of the soybean leaf improves the precision and accuracy in the estimation of leaf area using linear regression models. The shape of the leaflet, classified based on its circularity, avoids the subjectivity of its estimation. The hypothesis is confirmed, the morphology of leaflets and grains obtained with digital image analysis allows identifying differences in soybean varieties; therefore, it is useful for description and evaluation.

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