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Analysis of a Mini Core of Sesame (*Sesamum indicum* L.) Accessions Based on Seed Morphometric Data

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Abstract

Seed morphometric parameters (seed area, length, diameter, circumference, roundness and aspect ratio) of 15 accessions were analysed. Measurements on seeds were taken with help of a Computer- Camera system. Variation among accessions and genetic relationships were estimated. Great variability was observed for all parameters studied. High positive or negative correlation (r=0.726-0.963) was showed by Pearson's correlation analysis for the seed metric data of the 15 accessions. The cluster analysis revealed that the 15 accessions could be grouped into three to five clusters according to the methods (Single or Complete Linkage) used. The principal components analysis showed that the first two components accounted for 99.3% of the variability. Morphometric characterization of seeds is rapid, reproducible, reliable and cost effective. This technique of digital imaging is strongly recommended for researchers from developing country like Benin, because of its efficiency.

Keywords: *S. indicum* L., Seed size, Characterization, digital imaging

1. Introduction

Sesame (*Sesamum indicum* L.) is one of the oldest oilseed crop grown mainly for its seeds. According to Wei *et al*. (2015), sesame seeds have the highest oil content (40.83~61.88%) among major oilseeds. They also demonstrated that it was possible for breeders to generate sesame varieties with both high yield and high oil content. After the oil extraction, the residues obtained prove to be an importance source of protein for the food (Jefferson

Agricultural Institute, 2003). Human consumed almost exclusively sesame oil. In addition to its potential numerous industrial uses, Abhilash and Singh (2010) reported that sesame plants have also successfully been used for phytoremediation of lidane (organochlorine pesticides) from contaminated soils.

Sesame (*S. indicum* L.) accessions are at present characterized on the basis of plant morphological traits observed in the field. This method requires a relatively long time related to the duration of the vegetative cycle ranging between 80 and 160 days. Hence, there is a need to develop fast and reliable laboratory tests, being able to be used as alternative to field observations for the identification of sesame accessions. More than 96% of the world sesame seed are produced in Africa and Asia. The leading world producer's countries are India, China and Sudan in Africa (FAOSTAT, 2015).

Seed morphometric data have been used to evaluate accessions of *Phaseolus vulgaris* (Freytag *et al*., 2001; Marisol Lo Bianco *et al*., 2015). Digital image analysis offers an objective and quantitative methods for estimation of morphological parameters. With the evolution of imaging and computing hardware, several imaging systems have been developed for characterization and classification of many varieties of crops (Kehinde *et al*., 2017; Sandeep Varma *et al*., 2013; Divakara *et al.*, 2011). Since our previous work (Quenum, 2002), very few studies have been reported on the physical properties of the seeds of sesame. Some informations are available on the use of seed morphometric data for sesame germplasm analysis (Azeez and Morakinyo, 2011; Adebowale *et al.* , 2011, Prasad *et al*., 2013). This work has the aim to estimate biometric characteristics of seeds of a sesame mini-core collection through digital image analysis and use statistical means to identify and distinguish one accession to another; and subsequently to analyze variability to understand the diversity of seed morphometric traits.

2. Materials and Methods

2.1 Plant Material

Seeds of 15 accessions of sesame (Table 1) were investigated. About 50 undamaged seeds from each individual accession were taken at random and measured.

2.2 Measurements of on Seeds

For seeds measurements, the PC software Qwin and a 3CCD Colour Video Camera connected to the PC were used. This type of image analysis is a method which allows the determination of geometrical and grey scale values from digital images of arbitrary sources.

The parameters analysed were: seed area (mm^2), seed length (mm), seed width (mm), seed circumference (mm) and roundness (roundness is a factor which for a circle adopts the smallest value of 1. It corresponds to the square of an object's circumference, divided by its area). Aspect ratio : Ratio of length divided by width.

2.3 Statistical Analysis

Morphometric data were stored in MS Excel files. The analysis of variance (ANOVA) was applied to detect discriminant variables among genotypes. To find out the relevant variables for morphological seed description, a correlation matrix was built using Pearson correlation coefficients to aid in interpretation of the analysis, and thereafter a principal component analysis (PCA) was performed. The relationships between accessions were examined on the basis of six above parameters. All calculations were done using SAS 9.2 software and/or MINITAB (version 17 for Windows).

3. Results

Differences between accessions in all seed parameters (seed area, length, diameter, circumference, roundness and aspect ratio) were established (Table 2). The area of seed varied greatly among accessions from 2.91 mm^2 (ZZM2812) to 7.37 mm^2 (Chanzhongzhi). Accession Chanzhongzhi had the highest seed circumference (10.82mm), length (3.91mm) and width (2.45mm) while ZZM2812 had the lowest values. Roundness ranged from 1.16 in accessions ZZM2874 and Balinzhima to 1.45 in ZZM2737, which had the higthest aspect ratio (2.04) while accesssion ZZM2874 recorded the lowest value (1.47).

Significant and positive or negative correlation exists among all the seed morphometric parameters showed in Table 3.

Table 1. Name of the 15 accessions investigated in this study

Accession No.	Accession name	Accession No.	Accession name
1	ZZM0611	9	ZZM2737
2	ZZM1415	10	38-1-7
3	ZZM3186	11	ZZM2874
4	ZZM0795	12	Loahongzhulian
5	ZZM0829	13	Jioaganzhu
6	ZZM2431	14	Balinzhima
7	ZZM2812	15	Chanzhongzhi
8	ZZM2918	-	-

Table 2. Variation of seed morphometric parameters of sesame accessions using image analysis system

	Area(mm2)	Length(mm)	Width(mm)	Circ(mm)	Roundness	Aspect Ratio
Mean	5.60	3.44	2.13	9.51	1.24	1.64
Minimum	2.91	2.67	1.49	7.31	1.16	1.47
Maximum	7.37	3.91	2.45	10.82	1.45	2.04
Root-MSE	0.63	0.22	0.18	0.54	0.08	0.16
CV%	11.29	6.51	8.35	5.65	6.46	9.51
F-test	184.24 ***	90.47 ***	135.63 ***	131.33 ***	69.45 ***	45.88 ***
LSD.05	0.248	0.088	0.07	0.211	0.0315	0.0611

Table 3. Pearson Correlation coefficients of seeds morphometric traits in 15 accessions

Seed traits	Area	Length	Width	Circ	Roundness	Aspect ratio
Area	1					
Length	0.871***	1				
Width	0.932***	0.726***	1			
Circ	0.963***	0.932***	0.865***	1		
Roundness	-0.698***	-0.396***	-0.764***	-0.511***	1	
Aspect ratio	-0.533***	-0.124**	-0.755***	-0.377***	0.785***	1

NB: we used 50 seeds to estimate the correlation coefficients

The first two principal components for seed morphometric parameters revealed an account of approximately 99.3 % of the total variance in Table 4. The first principal component along accounted for 87.6% and the second 11.7%. The first component was largely and positively loaded with seed width (0.435), seed area (0.432) and seed circumference (0.419) and negatively loaded with seed roundness (-0.412). The second component had high negative loadings from seed aspect ratio (-0.684) and seed length (-0.529). These traits are important and adequate descriptors for seed traits study in this material.

Table 4. Eigenanalysis of the Correlation Matrix of seed morphometric parameters

Seed traits	PC1	PC2	PC3	PC4	PC5	PC6
Area	0,432	-0,158	-0,029	0,861	0,133	0,171
Length	0,391	-0,529	-0,070	-0,441	0,218	0,566
Width	0,435	0,064	0,273	-0,078	-0,850	0,060
Circ	0,419	-0,327	0,245	-0,145	0,228	-0,765
Roundness	-0,412	-0,341	0,817	0,136	0,025	0,163
Aspect ratio	-0,356	-0,684	-0,438	0,137	-0,400	-0,188
Eigenvalue	5.2536	0,6995	0,0379	0,0050	0,0032	0,0007
Proportion	0,876	0,117	0,006	0,001	0,001	0,000
Cumulative	0,876	0,993	0,999	0,999	1,000	1,000

The dendrograms shown in Figure 1and Figure 2 illustrate the relationships between the seeds of the 15 accessions of sesame. At total similarity, all accessions were different from each to other. In cluster analysis major clusters were found in respect to measurement using digital imaging technique. Single linkage clustering showed two major groups and a third comprising three accessions which did not correspond to any cluster (CV= 12.8~43.48%). At distance of 3.22, complete linkage clustering method showed five clusters. Seed of accession Chanzhongzhi appeared different from others, stand as outlier and formed along the Group 5 (Table 5).

Table 5. Traits mean variation between the seeds groups resulting from cluster analysis

Groups (accession number)	Cluster membership (accessions)	Area (mm2)	Length (mm)	Width (mm)	Circ (mm)	Roundness	Aspect Ratio
Single linkage							
Group1(10)	1,3,4,5,8,10,11,12,13,14	6,13	3,55	2,28	9,87	1,19	1,57
CV%		10,40	6,30	7,58	5,35	4,13	8,50
Group 2(2)	2 and 6	4,32	3,17	1,86	8,74	1,34	1,73
CV%		19,65	8,42	13,52	8,06	8,93	12,26
Group 3(3)	7,9 and 15	4,67	3,26	1,85	8,85	1,35	1,81
CV%		43,48	17,85	25,40	17,73	12,80	15,88
Complete linkage							
Group 1(5)	1, 4,5, 12, and 13	6,04	3,58	2,20	9,83	1,20	1,62
CV%		10,40	6,30	7,58	5,35	4,13	8,50
Group 2(5)	3,8,10,11,14	6,19	3,50	2,33	9,86	1,18	1,51
CV%		11,22	6,72	7,40	5,92	3,83	7,55
Group 3(2)	2 and 6	4,32	3,17	1,86	8,74	1,34	1,73
CV%		19,65	8,42	13,52	8,06	8,93	12,26
Group 4(2)	7 and 9	3,31	2,93	1,545	7,87	1,42	1,91
CV%		21,40	13,94	12,92	10,58	11,19	15,21
Group 5(1)	15	7,37	3,91	2,45	10,81	1,19	1,60
CV%		7, 56	4,63	6,82	4,19	3,12	7,13

CV: Coefficient of variation

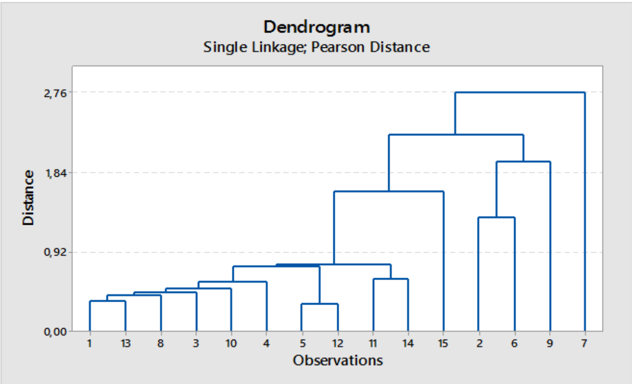

Figure 1. Dendrogram of 15 accessions using single linkage based on data generated from seed image

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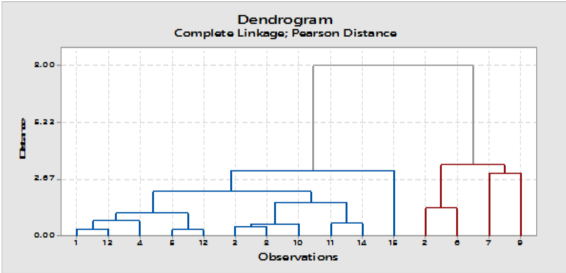

Figure 2. Dendrogram of 15 accessions using complete linkage based on data generated from seed image

4. Discussion

There are few attempts to use geometric morphometrics on sesame seeds proving that it can efficiently distinguish one from another accession.

4.1. Efficiency of Digital of Seed Morphometrics Techniques

In the present work, the digital geometric morphometric with camera captured analysis of the sesame seeds allows the discrimination of the 15 accessions of a mini-core collection. Seed area, Length, width, circularity and length width ratio are the key components of the seed size. Sandeep Varma *et al.* (2013) reviewed how to explore computer software-aided seed morphological details as an image analysis technique which can be applied to attain variety of purposes in seed science research. Digital image analysis (DIA) is the procedure in which digital image is transformed into digital values (Williams *et al.*, 2013). In this study, we have used 50 seeds per accession. Cui *et al.* (2011) have determined Kernel dimensions along with kernel length, kernel width and kernel diameter ratio with the help of a simple ruler. Similarly, previously kernel length (KL) and kernel width (KW) were determined by Vernier calipers (Ramya *et al.*, 2010). Without digital assistance, measurement of seed shape attributes becomes less precise and laborious. A software “Smart Grain” was developed by Tanabata *et al.* (2012) to analyse the digital images. That software also determines seven parameters: Area (A), Perimeter (P), Length (L), Width (W), Length to width ratio (L/W), Circularity (Cs) and Distance of Length-width intersection point to Centre of gravity (Ds) precisely and robustly (Muhammad, 2017). Recently, Ke Gao *et al.* (2017) presents a work on an image processing application named MUSeed, using an Android-based mobile Microscope, that automatically computes seed morphometry. From their work, area of each individual seed of an Arabidopsis is approximately 0.1mm^2. Emilio Cervantes *et al.* (2016) reported that seed image analysis based on geometric models might contribute to the botanical description of species, genus, or families and the identification and discrimination of genotypes, varieties, and species and the determination of diversity at inter- and intraspecific levels.

4.2. Morphological diversity of seeds and their relationships

Analysis of variance showed highly significant differences (P<.001) among *sesame* accessions for all parameters studied, indicating the existence of a high degree of morphological diversity of seeds. According to El Khier *et al*. (2008), the seed characteristics or physical properties of sesame seeds vary and this variation may likely be as a result of variability in genotypic effects. Emilio Cervantes *et al.* (2016) reviewed morphological variation in seed characters includes differences in seed size and shape. Seed shape is an important trait in plant identification and classification.

The range values of seed sizes in this study (length, width and ratio) are similar to those reported by Azeez and Morakinyo (2011). Our result for the six physical dimensions is more closer to those observed by Arafa (2007) in Egypt; Tunde-Akintunde and Akintunde (2004 and 2007) and Adebowale *et al*. (2010) in Nigeria.

In this study, correlation coefficients were generally high in among most of the seed morphometric parameters in both positive and negative (r=0.726-0.963) directions. This finding is in line with that of Daniel *et al.* (2012) who suggested that those parameters are potentially good quantitative taxonomic descriptions of maize seed morphological classification. According to Azeez and Morakinyo (2011), with high positive and significant correlations the improvement in one of these attributes will positively affect others in sesame and will consequently lead to increase in seed size. Kehinde *et al.* (2017) also reported similar result in *Hibiscus cannabinus*. Correlated quantitative traits are of a major interest in an improvement program, as the improvement of one character may cause simultaneous correlated changes in the other characters (Divakara *et al.*, 2011).

The result of principal component analysis and the dendrograms generated by the seed parameters reveal genetic relationship among the 15 accessions of sesame. The study of relationships is based on the assumption that the difference in the characters reveals their genetic divergence (Divakara *et al.*, 2011). In our present study, the clustering pattern showed that 15 accessions are grouped into 3 to 5 clusters based on the scores of first six principal components (PCs) derived from six seed traits, revealing geographical diversity which is not strongly related to genetic diversity. This absence of relationship between genetic diversity and geographical distribution is in accordance with the findings of our previous works in sesame using agro-morphological and molecular markers (Quenum *et al.*, 2004; Quenum and Yan, 2017). Similar results were reported by Kaushik *et al*., (2007b), Rao *et al*., (2008), Gohil and Pandya (2008) in *Jatropha curcas*; and Kaushik *et al.* (2007a) in *Pongamia pinnata*. Our current study also revealed few high coefficients of variation; while the remaining ones had values lower than 12% (Table 5). According to Andrés-Agustín *et al.*(2006), coefficients of variation of 12%, or less, are acceptable in the characterization of plant organs in horticultural species. This study used 50 seeds per accession, which showed continuous variables. Therefore, these numbers allow the use of ANOVA statistics and would not be desirable to increase the sample size if the coefficient of variation is higher.

Benin is a small sesame producer country in the Western Africa (FAO, 2000). Therefore, it is anticipated that commercial sesame cultivation will increase in this country. Komivi Dossa *et al*. (2016) indicated that West Africa was an isolated geographically gene pools for sesame. Thence, they suggested that future germplasm collections should focus on the accessions from this region. Furthermore, these new collections could be analysed by using digital imaging techniques.

5. Conclusion

Our results demonstrated that the digital image analysis allows the estimation of the principal dimensions of sesame seeds with high accuracy. The achievements allow demonstrating the usefulness of the discrimination system based on seed phenotypic characters, for the identification and classification of sesame accessions. The variables, Seed Area, Length, Width, Circumference, Roundness derived from image analysis, have a high discriminatory power. The availability of software for digital image analysis helps with the development of several indices. Considering the heterogeneous nature of the seed samples used in this study, the grouping of the accessions did not strong. Besides the analysis of morphological variables of seeds efficiency, molecular analysis remains an essential tool for the investigation of the variability within and between genotypes, and for estimating genetic relationships. Moreover, morphometric characterization of seeds is rapid, reproducible, reliable and cost effective. This technique is strongly recommended for scientists from developing country like Benin.

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