DE GRUYTER
OPEN

DOI: 10.2478/v10129-011-0085-z

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IMAGE-ANALYSIS BASED ON SEED PHENOMICS IN SESAME

ABSTRACT

The seed coat (testa) structure of twenty-three cultivated (*Sesamum indicum* L.) and six wild sesame (*S. occidentale* Regel & Heer., *S. mulayanum* Nair, *S. prostratum* Retz., *S. radiatum* Schumach. & Thonn., *S. angustifolium* (Oliv.) Engl. and *S. schinzianum* Asch) germplasm was analyzed from digital and Scanning Electron Microscopy (SEM) images with dedicated software using the descriptors for computer based seed image analysis to understand the diversity of seed morphometric traits, which later on can be extended to screen and evaluate improved genotypes of sesame. Seeds of wild sesame species could conveniently be distinguished from cultivated varieties based on shape and architectural analysis. Results indicated discrete 'cut off' values to identify definite shape and contour of seed for a desirable sesame genotype along with the conventional practice of selecting lighter colored testa.

Key words: phenomics, seed image analysis, sesame

INTRODUCTION

Sesame is the oldest oilseed plant used by humans (Weiss, 1983; Maberley, 1997). It was cultivated during Harappan, Mesopotamian, and Anatolian eras, and throughout the Greco-Roman world, both for its edible seed and for its oil. It was domesticated on the Indian subcontinent (Bedigian, 1984, 1998, 2000, 2003) though report of African origin of sesame is also available (Ihlenfeldt and Grabow-Seidensticker, 1979). It is possible that the genus *Sesamum* arrived on the Indian subcontinent at a very early period, before Gondwanaland broke apart, transferring sections of the genus to India. It was probably domesticated there later, somewhat after the intro-

duction of “founder” crops (Bedigian, 2003). Whatever is its evolution and/or centre of diversity, sesame is a crop of great importance, particularly to African and Asian farmers. Yet it is a case of typically hitherto neglected crop. It is not studied by any of the international agricultural research centers; and the paradigm of sesame parallels many minor crops: Sesame is not a major crop because there is little research and other way around there is little research on sesame because it is not a major crop (Prasad and Gangopadhyay, 2011).

Since sesame growers have been manipulating the crop due to migration and trade for centuries it has caused a steady gene flow among different geographical areas; and an assemblage of ‘wild’ and ‘derived’ traits was noticed in almost all genotypes in the phenomic analysis of seventy one accessions of Indian and exotic sesame (Prasad and Gangopadhyay, 2011). In the elegant and comprehensive review on evolution, domestication and diversity of sesame, Bedigian (2003) indicated three unique ‘wild’ traits viz. shattering of seeds, indeterminate growth and late maturity, which are still predominating in modern day cultivated sesames. Other changes (from wild to domesticated) in the crop that distinguish it from near wild relatives are seed and capsule characters. Since seeds are the objects of interest (in an oilseed crop), it makes sense that these are the prime focus of modification. Seeds of domesticated sesame are generally smooth, not rough-textured, with high oil content and display a diverse array of colors: the lighter colored ones are generally preferred for the purpose of direct consumption and the quality of extraction of oil since the seeds with darker seed coat (testa) often leave an undesirable stain in the oil. A critical study on phenomic analysis of testa would, therefore, be tempting to revisit the theory of domestication vis-à-vis to predict an improved genotype of sesame.

Image processing is a stimulating and an active field of research, where disciplines such as Engineering, Computer Science, Physics, Medicine and Biology inter-disciplinarily co-operate. An overview of the experimental integration between the standard germination test and a computer-aided image analysis system was first reported with the aim to investigate the potential of the new technique in monitoring seed imbibition phases and radicle elongation in broccoli, radish, lentil, lettuce and carrot seeds (Dell’Aquila, 2004), which was later further refined (Dell’Aquila, 2006). Development of a Seed Analyzer using the techniques of computer vision has been initiated recently to develop the method of identifying the best quality seeds by using the techniques of computer vision (Arya and Lehana, 2012).

The present phenomic study aims to analyze the testa structure of ten Indian, thirteen exotic accessions of cultivated sesame (*Sesamum indicum* L.) and six related wilds (*S. occidentale* Regel & Heer., *S. mulayanum* Nair, *S. prostratum* Retz., *S. radiatum* Schumacher & Thonn., *S. angustifolium* (Oliv.) Engl. and *S. schinzianum* Asch) with state-of-art image analysis software using the descriptors for computer based seed image analysis

(Dell'Aquila, 2004) to understand the diversity of seed morphometric traits, which later on can be extended to screen and evaluate improved genotypes of sesame through this approach.

MATERIAL AND METHODS

Material

The plant materials comprised of seed samples of twenty three accessions (ten Indian and thirteen exotic collections) of cultivated sesame (*Sesamum indicum* L.) and six allied wilds (*S. occidentale* Regel & Heer., *S. mulayanum* Nair, *S. prostratum* Retz., *S. radiatum* Schumach. & Thonn., *S. angustifolium* (Oliv.) Engl. and *S. schinzianum* Asch.). The twenty three accessions of cultivated sesame including Indian and exotic ones are the short listed genotypes, which showed desirable phenomic traits over a 'cut off value' of seventy, calculated by the algorithm developed on the basis of phenomic table of seventy one cultivated sesame to look for the best possible combination of colour code (represented by thirty traits of different polymorphic state) of the postulated 'target sesame' (Prasad and Gangopadhyay, 2011). Indian accessions were originally obtained from National Bureau of Plant Genetic Resources, India (NBPGR) while of the exotic collections, USDA accessions of *S. indicum* along with one wild (*S. prostratum*) were obtained through Dr. Edward J (Ned) Garvey, Plant Germplasm Quarantine Centre, BARC-East, Beltsville, Maryland, United States; and the Venezuelan germplasm of cultivated sesame (*S. indicum*) were kindly provided by Dr. Hernán E Laurentin, Biologic Sciences Department, Agronomy Faculty, Universidad Centroccidental Lisandro Alvarado, Barquisimeto, Venezuela. Seeds of the other wild sesames (viz. *S. occidentale*, *S. mulayanum*, *S. radiatum*, *S. angustifolium* and *S. schinzianum*) were kindly provided by Mr. K Masuda, Department of Biology, Faculty of Science; University of Toyama, Japan. The particulars of all the germplasm are tabulated in Table 1.

Table 1

Particulars of the sesame germplasm under study

Indian(NationalBureauofPlantGeneticresources,NBPGR)accessionsof <i>Sesamumindicum</i>							
Variety/ Accession No.	Abbreviations used	State of collection	Seedparametersstudied				
			Seed coat texture	Seed coat colour	Seed shape	Mean ($\pm$ SE) seed length [μ] (n=5)	Mean ($\pm$ SE) seed width [μ] (n=5)
1	2	3	4	5	6	7	8
IC131577	NBM02	Maharashtra	Smooth	White	Oval with concave side	3013.12 $\pm$ 42.79	1667.43 $\pm$ 40.39
IC131726	NBM07	Maharashtra	Partially reticu- lately rough	Cream	Oval with concave side	3500.14 $\pm$ 34.60	1803.67 $\pm$ 73.37
IC131992	NBMP01	Madhya Pradesh	Smooth	Dark Brown	Oval with concave side	2986.02 $\pm$ 80.55	1698.75 $\pm$ 77.57
IC131989	NBMP02	Madhya Pradesh	Smooth	Light Brown	Oval with concave side	2604.69 $\pm$ 82.29	1515.35 $\pm$ 36.69
IC131860	NBR01	Rajasthan	Partially radially rough	Medium Brown	Oval with concave side	2031.63 $\pm$ 69.48	1030.19 $\pm$ 29.52
IC131987	NBR02	Rajasthan	Smooth	Cream	Oval with concave side	2549.87 $\pm$ 49.23	1419.25 $\pm$ 45.71
IC96038	NBUP01	Uttar Pradesh	Smooth	Cream	Oval with concave side	2379.90 $\pm$ 37.61	1407.35 $\pm$ 35.21
IC131630	NBG01	Gujarat	Partially rough	Dull Black	Oval with concave side	2190.50 $\pm$ 64.13	1228.60 $\pm$ 34.08
IC132114	NBP01	Punjab	Radially rough	Cream	Oval with concave side	2514.61 $\pm$ 65.52	1491.51 $\pm$ 41.70
IC131690	NBTN01	Tamil Nadu	Smooth	Cream	Oval with concave side	2497.13 $\pm$ 68.78	1340.28 $\pm$ 30.30
UCLA295	VN02	Venezuela	Smooth	White	Oval with concave side	2894.93 $\pm$ 52.91	1632.06 $\pm$ 40.75
UCLA37	VN04	Venezuela	Partially rough	White	Oval with concave side	2848.73 $\pm$ 13.36	1631.41 $\pm$ 59.47
UCLA65	VN05	Venezuela	Smooth	White	Oval with concave side	2027.50 $\pm$ 39.26	1317.17 $\pm$ 10.66
UCV3	VN10	Venezuela	Partially rough	Dark Brown	Oval with concave side	2250.70 $\pm$ 50.03	1321.88 $\pm$ 89.92
PI175907	UST09	USDA/ Turkey	Smooth	Medium Brown	Oval with concave side	2529.34 $\pm$ 61.39	1515.11 $\pm$ 38.90
PI170730	UST12	USDA/ Turkey	Smooth	Tan	Oval with concave side	2847.07 $\pm$ 27.53	1661.81 $\pm$ 47.01
PI170733	UST13	USDA/ Turkey	Partially rough	Grey	Oval with concave side	2889.19 $\pm$ 48.21	1768.54 $\pm$ 39.47
PI173960	USIN03	USDA/ India	Smooth	Cream	Oval with concave side	3030.05 $\pm$ 79.95	1763.56 $\pm$ 63.24
PI195122	USC02	USDA/ China	Partially rough	Medium Brown	Oval with convex side	2714.88 $\pm$ 109.49	1510.74 $\pm$ 47.12

Table 1

Continued							
1	2	3	4	5	6	7	8
PI195123	USC03	USDA/ China	Partially radially rough	Light Brown	Oval with concave side	3017.57 ±61.07	1952.71 ±42.01
PI198157	USIR02	USDA/Iraq	Smooth	Light Brown	Oval with concave side	3088.85 ±36.67	1860.07 ±63.93
PI198158	USSU01	USDA/ USSR	Partially rough	Dark Brown	Oval with concave side	2874.57 ±27.95	1649.15 ±52.53
PI200109	USSU02	USDA/ USSR	Partially rough	Dull Black	Oval with concave side	2776.55 ±91.46	1635.80 ±49.49
<i>S. occidentale</i>	S. occ	Japan	Partially rough	Medium Brown	Oval with concave side	2992.07 ±48.86	1939.55 ±62.96
<i>S. mulayanum</i>	S. mul	India	Reticulately rough	Dull Black	Oval with concave side	2390.44 ±56.49	1710.76 ±55.87
<i>S. prostratum</i>	S. pros	USDA/ India	Partially rough	Cream	Oval with concave side	2580.99 ±82.64	1845.58 ±26.70
<i>S. radiatum</i>	S. rad	Cameroon	Partially radially- rough	Tan	Oval with concave side	2190.45 ±71.00	1542.66 ±57.82
<i>S. angustifolium</i>	S. angust	Japan	Reticulately- rough	Dull Black	Oval with concave side	<u>1892.07</u> <u>±73.45</u>	1240.42 ±42.26
<i>S. schinzianum</i>	S. schinz	Myanmar	Partially radially rough	Dark Brown	Elongated	2660.98 ±61.37	1756.21 ±51.45

Image acquisition of seeds

Digital images of the germplasm were taken from uniform seed population (maintained in field for successive years to obtain pure line) with KODAK EASY-SHARE ZD710 ZOOM Digital Camera as JPEG, which later on converted to TIFF prior analysis through software. Seeds of selected germplasm were subjected to Scanning Electron Microscopy (SEM) after sputter coating with gold (7 mA). Images (in TIFF) were captured under FEI Quanta 200 MK2 Scanning Electron Microscope.

Image analysis software and descriptor for analysis

Images of seeds (captured either through Digital Camera or SEM) were analyzed with Image-Pro[®] Plus software (Version 7.0 for Windows[™]) of Media Cybernetics, Inc. After acquirement of the images as TIFF, the respective magnification was calibrated since by default, the software expresses all spatial measurements in terms of pixels. The spatial scale was changed accordingly to make measurements in terms of microns for the sake of convenience. The parameter of initial analysis for all the germplasm was measurement of mean seed length and width, which was performed from the images captured with digital camera. After necessary calibration, using the 'Best fit Line feature' of 'Measure' menu the seed length and widths were derived from five random seeds of the image containing population of seeds.

The mean value with necessary statistical analysis was considered (Table 1). Images of SEM of selected germplasm were initially calibrated as stated earlier followed by using the command of 'count/size command' from the 'Measure' menu of the software and the descriptor for computer based seed image analysis (Dell'Aquila, 2004) was mainly followed to measure the following parameters: Area, Aspect, Perimeter, Roundness and Radius (Table 2). Furthermore, the surface plots of the seeds from SEM images were also generated following the software manual.

Table 2

Parameters under study: Descriptor for computer based seed image analysis (following Dell'Aquila 2004 and the manual of Image-Pro® Plus software (Version 7.0 for Windows™))

Parameter	Unit	Definition and illustration
Area	micron ²	<p>The area of each object (minus any holes). The area comprised of pixels having intensity values within the selected range is reported. An object is considered within a site if its centre of gravity lies within the site outline.</p>
Aspect	-	<p>The ratio between the major axis and the minor axis of the ellipse equivalent to the object (i.e., an ellipse with the same area, first and second degree moments), as determined by Major Axis/Minor Axis. Aspect is always greater than 1.</p>
Perimeter	micron	<p>Measurement to report the length of the outline of each object using a polygonal outline. The perimeter of interior holes is not included in this measurement.</p>
Roundness	-	<p>The roundness of each object, as determined by the following formula: $(\text{Perimeter}^2) / (4\pi\text{Area})$. Circular objects will have a roundness = 1; other shapes will have a roundness > 1.</p>
Radius	micron	<p>The maximum distance between each object's centroid pixel position and its perimeter.</p>

Construction of phenogram

Fig. 1. The neighbor-joining representation of phenogram of twenty-three cultivated and six wild Sesame genotypes constructed with software DARwin 5.0.128 showing clusters/sub clusters based on the morphometric seed characteristics. Representative digital images of the germplasm taken from uniform seed population are placed as insets. The * marked genotypes were further analyzed and results are presented in Figures 2 and 3. The arrow marked genotype (USC02) was found to be an out group.

A table of character state was developed based on three qualitative (seed coat texture, colour and shape) and two quantitative (seed length and width) morphometric parameters of twenty three accessions of cultivated and six wild sesames. Standard descriptor of sesame (NBPGR and IPGRI) was followed for the qualitative traits and numeric was assigned for each and every character states under study. The mean data ranges of the quantitative parameters (Table 1) were arranged in continuous class intervals and numeric was assigned accordingly to each class interval. The table of character state was imported to dedicated statistical software (DARwin 5.0.128) and subsequent analysis was done considering continuous dissimilarity data to perform weighted neighbor-joining tree (WPGMA, graphical representation of cluster analysis) based on Euclidean distance calculation (with Bootstrapping). Representative portions of digital images of the germplasm taken from uniform seed population were placed as insets in the respective positions of the phenogram (Fig.1).

RESULTS

Seed morphometric parameters

Seed coat (testa) texture varied from reticulately rough to smooth at two extremes. The wild sesames showed conspicuous testa roughness of different level (viz. partially rough, partially radially rough and reticulately rough) following the descriptor, while the germplasm of cultivated sesames exhibited smooth testa in general though there were occurrences of partial roughness in certain germplasm. Of the maximum polymorphic character state (ten in descriptor), eight was noted in the qualitative trait testa colour in the germplasm under study; and the general trend was lighter colored testa in the cultivated sesames while the darker shades predominated in the wild sesames (excepting *S. prostratum*). Shape of seed, the third qualitative trait under study, is represented by three character states in the descriptor and of these three almost all germplasm fell under two categories, viz. oval with concave side or oval with convex side with the exception of one wild sesame *S. schinzianum*, which showed elongated seed shape (Table 1).

Results of two quantitative (seed length and width) parameters as obtained from image analysis software were considered as continuous variable and grouped in nine class intervals of 200 μ considering the standard deviation from mean of the data. Length of seed varied from 3500 to 1892 μ while width of seed varied from 1952 to 1030 μ . Maximum length of seed ($3500.14 \mu \pm 34.60$) measured was in case of the cultivated sesame (*S. indicum*) accession NBM07, an Indian accession, while *S. angustifolium*, a wild sesame, showed least seed length ($1892.07 \mu \pm 73.45$). Widthwise the value of USC02, a Chinese accession of cultivated sesame was the maximum ($1952.71 \mu \pm 42.01$), while the width was minimum ($1030.19 \mu \pm 29.52$) in case NBR01, an Indian accession of cultivated sesame (Table 1).

Phenogram

Cluster analysis based on the aforesaid five seed parameters (three qualitative and two quantitative) was performed and WPGMA neighbor joining phenogram demarcated twenty nine genotypes (twenty three cultivated accessions of *S. indicum* and six wild sesames) into one large cluster (with two conspicuous sub clusters), one small cluster and an out-group. The out-group was represented by one Chinese genotype (USC02), maintained in USDA repository. The large cluster consisting of twenty germplasm was found to be evenly secluded into two sub-clusters, each having ten genotypes. The sub cluster 1 consisted of seven cultivated sesame accessions (*S. indicum*), three from Indian (NBMP01, NBM07 and NBM02) and four from USDA repository (USSU01, USC03, USIR02 and USIN03) of four different countries; two Venezuelan genotypes

(VN02 and VN04) and one wild sesame (*S. occidentale*). Of the ten genotypes clubbed in sub cluster 2 the presence of four wild sesames (*S. schizianum*, *S. radiatum*, *S. mulayanum* and *S. angustifolium*) was noted along with six cultivated sesame accessions (two from Indian repository, viz. NBR01 and NBG01, three from USDA repository, viz. UST12, UST13 and USSU02 and one Venezuelan VN10 germplasm). The smaller cluster consisted of seven cultivated sesame accessions of which five were from Indian repository (NBMP02, NBP01, NBR02, NBTN01 and NBUP01), one from USDA repository (UST09), one from Venezuela (VN05); and one wild sesame (*S. prostratum*) (Fig.1).

The relative proximity vis-à-vis distantness between the germplasm was further evident from the inset images of seed population. The sub cluster 2 of the large cluster comprised predominantly of darker colored testa while a gradation of light brown to whitish coloration was prevalent in the seeds of germplasm of sub cluster 1. The later trend was also noticed in cluster 2 (Fig.1).

Analysis of SEM images on image based descriptor

Representative seeds (thirteen; seven accessions of *S. indicum* and six wild sesames) selected from the phenogram were subjected to scanning electron microscopy (marked in Fig. 1) and the images were analyzed on the basis of seed image analysis descriptors. Of the five pertinent parameters analyzed, the relative values of area and roundness were co relatable in case of wild and cultivated sesames (figures 2A, B). In general, the wild sesames (except *S. angustifolium*) showed higher surface area than the cultivated ones. The highest surface area was represented by *S. occidentale* (455795 micron²) followed by *S. prostratum*, *S. schizianum*, *S. mulayanum* and *S. radiatum* in the descending order. All the *S. indicum* accessions showed low seed surface area (within the range 154136 to 126379 micron²) (Fig. 2A). The parameter, roundness of seed was determined by the formula: $(\text{Perimeter}^2) / (4\pi\text{Area})$ with the deduction that a circular object will have a roundness = 1; other shapes will have a roundness > 1. Following this assumption it was observed that all the wild sesames (with the exception of VN05, cultivated sesame) showed near roundness (value ranging between 1.20 to 2.01); maximum roundness exhibited by the seeds of *S. radiatum*. The cultivated sesames, on the other hand, showed greater roundness values (ranging between 5.32 to 2.47) indicating their deviations from circular object outline; maximum deviation from circular object was exhibited by the seeds of VN02 (Fig. 2B).

Fig. 2A. Histograms (with trend line equation and regression values) of seed image (Scanning Electron Microscope) analysis (Image-Pro® Plus software) derived data of area (

Fig. 2B. Histograms (with trend line equation and regression values) of seed image (Scanning Electron Microscope) analysis (Image-Pro® Plus software) derived data of roundness

Fig. 2C. Histograms (with trend line equation and regression values) of seed image (Scanning Electron Microscope) analysis (Image-Pro® Plus software) derived data of radius

Fig. 2D. Histograms (with trend line equation and regression values) of seed image (Scanning Electron Microscope) analysis (Image-Pro® Plus software) derived data of aspect

Fig. 2E. Histograms (with trend line equation and regression values) of seed image (Scanning Electron Microscope) analysis (Image-Pro® Plus software) derived data of Perimeter

The trend of the data of radius of object (seed) was similar to that of surface area since four wild sesames (*S. occidentale*, *S. prostratum*, *S. schizianum* and *S. mulayanum*) revealed greater value of radius (ranging between 561.71 to 470.14 micron) while the cultivated sesames showed smaller radius (ranging between 384.64 to 329.24 micron) (Fig. 2C). However, against this general observation, the wild sesame *S. angustifolium* showed the least radius (307.98 micron) measurement. Similar trend of data between roundness and aspect ratio was observed as high aspect values (the ratio between the major axis and the minor axis of the ellipse equivalent to the object) were noticed in case of cultivated sesames while the wild ones showed low aspect values; the maximum and minimum being 1.880 (VN02) and 1.332 (*S. angustifolium*) respectively (Fig. 2D). The perimeter of the seeds showed a wide range of values with maximum and minimum being 3383.91 (*S. schizianum*) and 1529.48 micron (VN05) respectively (Fig. 2E).

Surface plots of seeds

The 'True Color images' (RGB values in the intensity scale 0 to 255) of the Scanning Electron Microscopy photographs of selected seeds, after necessary processing through steps of software (e.g. assignment of automatic bright / dark objects for measurement) were subjected to 'surface plot' for an understanding of the relative contour of the objects (seeds) under study. Smoothest contour was noticed in case of seed of *S. prostratum*, followed by *S. indicum* accession

VN05; while the seeds of wilds like *S. angustifolium*, *S. radiatum* and *S. mulayanum* showed species specific surface plots. The exotic accession of *S. indicum*, USC02 showed an intermediate surface contours between wilds and cultivated sesame accessions (Fig.3).

Fig. 3A. Analysis of surface contours (Image-Pro® Plus software) of representative Sesame seeds. SEM photographs of *S. angustifolium* (a), *S. radiatum* (d), *S. mulayanum* (g), *S. prostratum* (j), *S. indicum* (USC02) (m), *S. indicum* (VN05) (p); assignment of automatic bright / dark objects for measurement in the respective images: (b), (e), (h), (k), (n) and (q); surface plots of respective images: (c), (f), (i), (l), (o) and (p)

Fig. 3A. Analysis of surface contours (Image-Pro® Plus software) of representative Sesame seeds. SEM photographs of *S. angustifolium* (a), *S. radiatum* (d), *S. mulayanum* (g), *S. prostratum* (j), *S. indicum* (USC02) (m), *S. indicum* (VN05) (p); assignment of automatic bright / dark objects for measurement in the respective images: (b), (e), (h), (k), (n) and (q); surface plots of respective images: (c), (f), (i), (l), (o) and (p)

DISCUSSION

Biological life, including seeds, has been classified using the Linnaean system since the mid-1700s. Seed coat patterns appear to mark different evolutionary levels inside of many taxonomic groups and variation of the testa characters is sufficient to distinguish taxa at sectional level (Celep *et al.*, 2012). A renewed interest in this area of research both from phylogenetic vis-à-vis applied (agricultural) points of view has probably ‘sown the seed’ of development towards ‘Metadata’ for plant seeds (Wilson, 2009). A boom in molecular techniques in recent times has targeted this research heavily towards the direction of seed DNA based genotyping for cultivar identification and development through molecular marker assisted selection (Gao *et al.*, 2008). While the term genomics refers to the characterization of the DNA constitution of an organism,

the term phenomics refers to the characterization of the phenotypic or observable traits of the organism. There is intense interest in phenomics, which refers to the development of efficient, high-throughput methods to determine phenotypes of many individuals for multiple traits, especially applicable in case of seeds (<http://www.seedquest.com/keyword/seedbiotechnologies/primers/varietydevelopment/phenomics.htm>). Analysis of digital and SEM images of seed testa of cultivated and wild sesames through state of art software, hence, was performed in the present study to understand the diversity of seed morphometric traits, the deduction from which later on can be extended to screen and evaluate improved genotypes of sesame.

The rationale of using the qualitative (from plant descriptor) and quantitative (from image analysis based descriptor) traits was evident from the results of the phenogram constructed on the basis on the aforesaid morphometric trait based parameters (Fig.1); coupled with placing of the representative portions of digital images of the germplasm taken from uniform seed population as insets in the respective positions of the phenogram. A clear demarcation of the seeds of the germplasm under study was noticed and the colour of the testa, one of the qualitative traits as selection criterion, probably proved its credential supporting the hypothesis of domestication as well as the common practice of selecting the seeds of lighter colored testa for cultivation to obtain good quality of oil. The gradual shifting of darker colored testa to the lighter one was evident in the cluster 2 and sub cluster 1 of the cluster 1 in the topological form of the WPGMA neighbor-joining tree form of cluster analysis. The end resultants of the phenogram are the accessions of the cultivated sesame (viz. VN02 and NBM02 in the case of sub cluster 1 of the cluster 1 and VN05 and NBUP01 in case of cluster 2), which are of lighter colored seed testa and also are the desired genotypes above the 'cut off value' (Prasad and Gangopadhyay, 2011). The members of the sub cluster 2 of cluster 1, on the other hand, were predominantly with darker colored testa and four wild sesame species have been clubbed in that sub cluster (Fig.1). The explainable trend of clustering of the germplasm on the basis of morphometric parameters, plausibly was due to the fact that the traits under study were of both qualitative and quantitative in nature; and the quantitative parameters, viz. length and width of seeds were measured through state-of-art image analysis software, which resulted in precision data that could have not been possible through manual means, particularly for the case of sesame seeds of very minute dimensions. The role of software aided image processing to gather precision data is becoming popular day by day as a number of leaf dimension parameters including height, width, perimeter and area have been measured and analyzed with highest accuracy (Ali *et al.*, 2012). The out-group of the phenogram represented by the Chinese genotype (USC02), maintained in USDA repository seemed to be unique cultivated sesame (*S. indicum*) with distinctive

traits since it also showed an intermediate surface contours between wilds and cultivated sesame accessions as revealed in 'surface plot' (Fig.3). The further uniqueness of this genotype was the occurrence of multiple pods per axil (Prasad and Gangopadhyay, 2011). It remains to be seen whether the aforesaid trait has some association with its unique seed contour, which probably would give a phenomic selection tool that can be applied to screen desirable genotypes with multiple pods, indicative of higher yield.

Shape of seed vis-à-vis its geometry though is an active area of research but the major bottleneck is to answer the question "Can you provide accurate descriptions of seed shape?" (https://www.researchgate.net/project/Description_of_seed_shape/). Geometrical analysis (elliptic Fourier transform method) was applied to grapevine seed outlines from modern wild individuals, cultivars and well-preserved archaeological material from southern France, dating back to the first to second centuries to understand seed domestication syndrome and reveal origins of ancient European cultivars (Terrall *et al.*, 2010). In the present endeavor with cultivated and wild sesame germplasm certain parameters on the basis of descriptors for computer based seed image analysis (Dell'Aquila, 2004) were analyzed with a futuristic approach to look for certain phenomic markers, which can later be applied to screen novel genotypes of sesame in breeding program. A gradual transition from circular to ellipsoid nature of cultivated sesame accessions was observed in the present study (Fig. 2B) since most of the wild sesame species showed near roundness of the seed outline. Diversity of legume seeds ranging from circular to cardioids (the trajectory described by a point of a circle that rolls around another fixed circle with the same radius) has been studied by Cervantes *et al.* (2012) through computational mathematics – geometric analysis with the observation that an elongation by a factor of ϕ in the vertical direction produced the 'best fit' cardioids from circle (round shape, considered as unmodified cardioid). With the assumption that the circular shape is the 'primitive' or 'wild' form (the argument though is a circular one; that form is present in the wild sesames and stating otherwise circle is a relatively simpler form) and altered seed shape and size is the resultant of human selection and domestication (Wang and Chee, 2010), the results of present study are explainable. Geometrically both a circle and an ellipse are closed curved shapes. However, in a circle, all points on the circle are equidistance from the centre, while instead of having all points the same distance from the centre point, an ellipse so shaped that if the distances from foci of the ellipse are added together, same number results. The values of aspect, one of the parameters under study, which is the ratio between the major axis and the minor axis of the ellipse equivalent to the object, was found to be always on the higher side in case of the cultivated sesame accessions, thereby substantiating the observation of their more inclination towards ellipse or ellipsoid structures, due to the

process of domestication and constant human selection for high yielding larger seeds. Curiously enough, the values of relative areas were greater in case of the seeds of wild *Sesamum* spp. in respect to the cultivated ones, though dimensionally most of the former ones were smaller (with the exception of *S. occidentale*) (Fig. 2A, Table 1). This apparent anomaly can be attributed to the surface contours of most of the wild sesames (Fig.3), where the unique ornamentation of the testa ultra structures resulting in conspicuous ridges and furrows (in *S. angustifolium*, *S. radiatum*, *S. mullayanum*) have contributed to the higher values of area.

It can be concluded that the present software aided image analysis approach applied to sesame seeds from wild species and cultivars allow the quantification of patterns of different qualitative morphological parameters amenable to mathematical interpretation. Seeds from wild sesame species can conveniently be distinguished from cultivated varieties based on shape and architectural analysis. Finally, to screen and evaluate an improved genotype of sesame with this approach it can be indicated that following parameters with respective 'cut off' values can be utilized: Shape of seed (ellipsoid with roundness factor >3, aspect value >1.5) and contour of seed (in the smoother direction) along with the conventional practice of selecting lighter colored testa.

ACKNOWLEDGEMENTS

Authors are thankful to Mr. Amaresh Chowdhury, ME and Mr. Raj Banerjee, BCE for deduction of mathematical data. The technical assistance of Mr. Subhas Maikap and Dr. Srikanta Chakraborty are thankfully acknowledged for SEM. The suggestions of Professor Rabin Majumder regarding improvement of quality of English of the manuscript are gratefully acknowledged. The financial assistance in form of research fellowship (3/1/3/JRF-2010/MPD-41 (32337)) provided by ICMR, India is acknowledged by one of the authors (RP). Authors are also grateful to the Director, Bose Institute.

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