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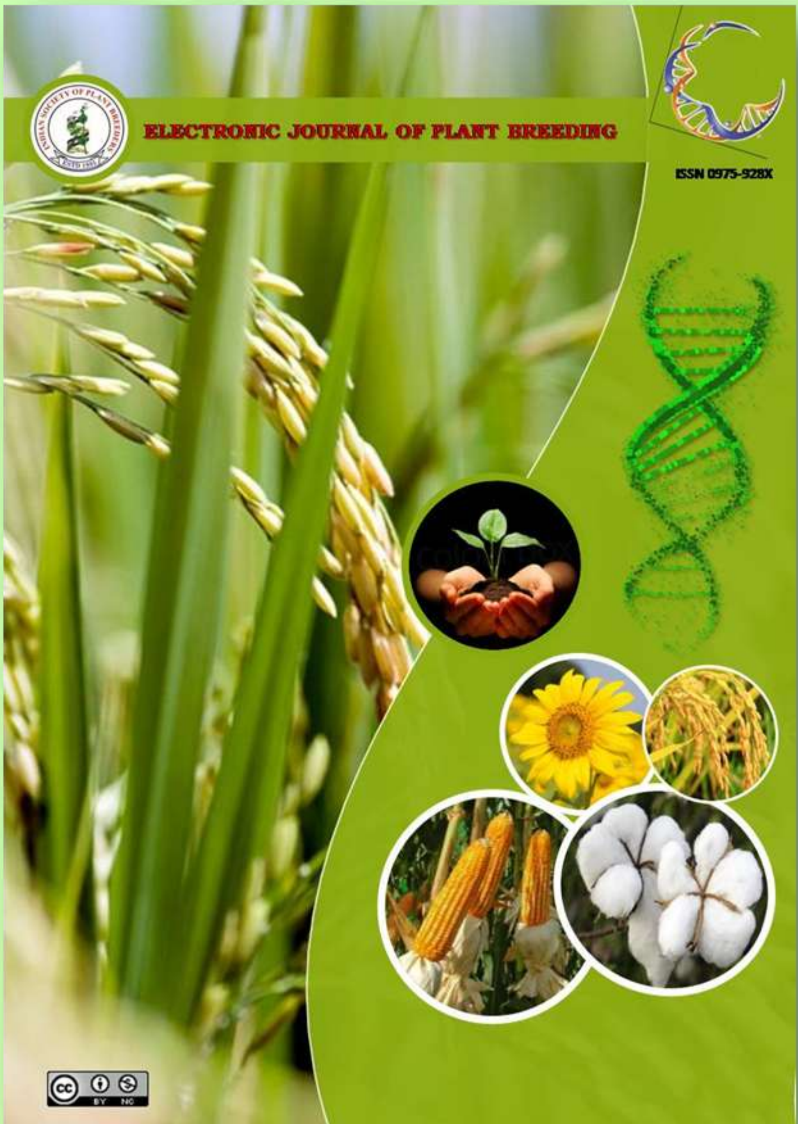

ISSN: 0975-928X

Volume: 10

Number:2

EJPB (2019) 10(2):785-790

DOI:10.5958/0975-928X.2019.00104.2

https://ejplantbreeding.org

Electronic Journal of Plant Breeding, 10 (2): 785-790 (Jun 2019) DOI: 10.5958/0975-928X.2019.00104.2
ISSN 0975-928X

Research Article

Characterization of sesame varieties through image analysis

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(Received:13 Mar 2019; Revised:26 Apr 2019; Accepted:11 Jun 2019)

Abstract
The identification and discrimination of crop varieties is very crucial for their breeding, variety registration, seed production and certification. Oligogenic morphological characters of seeds are distinct as well as stable and hence can be used for identification of varieties. Digital image analysis is an alternative to the manual classification of biological seed by integrating an image acquisition device and a computer. Data collected on sesame seed characters using grain scanner has significant differences for every observation. The sesame variety, SVPR 1 recorded the highest seed surface area (3.92mm^2), perimeter (7.88mm) and length (3.32mm) when compared to all other varieties. Cluster analysis revealed that the varieties could be grouped into two major clusters in which CO 1, TMV 3, TMV 4, TMV 5, TMV 7 formed one cluster whereas the other varieties were grouped under another cluster, which showed that the genotypes in one cluster had similarity in most of the parameters and also its parentage. Thus, image analysis helps in discriminating the morphological variation in seeds related to genotype and its evolution.

Key words
Sesame, Discrimination, Morphological characters, Surface area, Perimeter, Cluster analysis.

Introduction

Plant variety and cultivar identification is one of the most important aspect in agriculture. The identification and discrimination of crop varieties is very crucial during varietal development, variety registration, seed production, certification and quality control programmes. It plays a crucial role in all stages of seed production chain in order to avoid varietal deterioration. Distinctness Uniformity and Stability (DUS)characterization of plant and seed morphological traits is very critical for the proper implementation of Plant Varietal Protection (PVP) and Plant Breeder Rights (PBR) programs in India. Oligogenic morphological characters are distinct as well as stable and hence can be used for discrimination of varieties. The traditional methods for seed quality assessment are tedious, time consuming, and inherently inconsistent. Alternatively, an efficient and cost-effective instrumentation system is available to differentiate seeds of other varieties from a seed lot.

In the present decade, many researchers have made an effort to identify non-destructive methods for identification of cultivars during seed quality control programmes. Seed image analysis has significant use in determining the cultivar identity of seed lots and testing of the genuineness of new cultivars for the registration of new varieties .Seed quantitative descriptors generated by digital imaging analysis had been found to better define cultivar identity in comparison to traditional sensorial descriptors which are categorical descriptors (Dell' Aquila, 2006). Jayas *et al*. (2000) proved that the digital image analysis is a substitute to the manual classification of biological seed by integrating an image acquisition device and a computer.

Venora *et al.* (2009)opined that identification of Italian landrace of bean by using image analysis system contributed for cataloging and conservation of different land races and could be used in germplasm bank or *ex-situ* conservation. Dana *et al.*(2008) used computer image analysis to differentiate flax cultivars and recommended that current qualitative seed descriptors continuously used for cultivar characterization may be augmented by more descriptive quantitative descriptors which can be obtained at low cost from dry flax seeds. Keeping these highlights, an attempt was made with seed morphological traits to characterize the varieties of sesame using image analysis system.

Materials and Methods

The genetically pure seeds of different sesame varieties viz., CO 1, SVPR 1, TMV 3, TMV 4, TMV 5, TMV 6, TMV 7, VRI 1, VRI 2and VRI 3 were collected from various research stations of Tamil Nadu Agricultural University. The laboratory experiment was carried out at Department of Seed Science and Technology and Centre of Innovation, Agricultural College and

Research Institute, TNAU, Maduraiusing Satake RSQI10A Grain Scanner. The seeds were scanned with Charger Coupled Device (Color) sensor with LED white light source. Before going for actual measurements, self-diagnosis was made by placing blue background plate and sample tray. Then, ten seeds were placed in five replications on the sample tray and the seeds were scanned. Parameters like seed area, length, width, perimeter, aspect ratio, circularity, rectangularity were obtained for individual seeds in SATAKE software package.

Surface area is a measure of the size of plane taken by an object, and it is a metric evaluation for object size in the two-dimensional space (Sacilik *et al*. 2003). It is expressed in mm^2.Width is the maximum width measured perpendicular to straight length and length is the straight distance between two points stretching from the base of the embryo axis to the tip of the endosperm of the seed whereas perimeter is the axis of a circle drawn around the seed touching all edges. All these parameters were expressed in mm.

Ratio between the length and breadth of the seeds was expressed as Aspect Ratio. Balkaya and Odabas (2002) refer to this magnitude as the Eccentricity Index. Circularity index is calculated as suggested by Rovner *et al.* (2007)which is a measure of the similarity of a plane figure to a circle. It ranges from 0 to 1 giving the value of one for circles and it is a useful magnitude as a first approximation to seed shape. Rectangularity indicates the external smallest rectangle of target object and reflects the filling degree. The object placement angle has great influence on external rectangle.

The data obtained from grain scanner were analysed by the ‘F’ test of significance following the methods described by Rangaswamy (2002). The critical differences (CD) were calculated at 5 per cent probability level. The data were tested for statistical significance. If the F test is non-significant, it was indicated by the letters NS. Cluster analysis was also made using STAR 2.0 statistical software package released by IRRI.

Results and Discussion

The machine vision system through image analysis is effectively utilized for cultivar discrimination and has greater hope in testing the distinctness of new varieties for registration (Van de Vooran *et al.* 1991). The physical parameters like seed size shape, perimeter and other related parameters are clearly discriminating the seeds of different varieties and hence data on the seed morphological traits were observed using grain scanner utilizing ten different varieties of sesame. In the present study, the data on individual seed area and perimeter (Table 1)revealed that the largest seed was SVPR 1 (3.92mm) followed by VRI 3 (3.64mm). The variety TMV 5 recorded the smallest surface area (3.19mm) when compared to other varieties. Wyille-Echeverria *et al.* (2003)reported that seed size and shape showed difference among *Zostera marina* cultivars. These variations might be due to difference in the length and width of the seed. SVPR 1 recorded the highest seed length (3.32mm) and width (1.68mm) which paves the path for attaining the maximum area and perimeter. TMV 5 possesses the lowest seed length (3.03mm) and width (1.52mm) which resulted in smaller seed area and perimeter. Shape of VRI 3 was almost circular (0.81) because of broader width (1.72mm) and shorter in length which results in the reduction of seed area and perimeter than SVPR 1.

Tanabata *et al.*(2012) recorded physical parameters of seeds through image analysis by using Smart Grain-high throughput phenotypic software and conducted QTL analysis for seed shape and found discriminations among seed shape and size. Aspect Ratio *i.e.* the ratio between major axis and minor axis was found to be significantly influenced by different varieties. It was maximum in TMV 3 (2.06) followed by CO 1, TMV 4, and TMV 5 and minimum in VRI 3 (0.81) indicating the circular nature of seeds (i.e.) width was more in VRI 3. The rectangularity of the seeds was recorded the highest in SVPR 1 (3.13) due to the highest seed length. Adewala *et al.* (2010)observed the seed area, length and perimeter of African yam bean seeds and found more variability in their seed metric variables of different genotypes which would be very useful for effective discrimination of inbreds and their hybrid derivatives.

The variation in each factor might be due to the genotypic influence which confirmed genotypic variations in seed morphology. Sahoo *et al.*(2000)observed differences among seeds of sunflower varieties using machine vision approach. Anouar *et al.*(2001)grouped different varieties of carrot seeds using image analysis technique. By using image analysis technique, varietal identification and characterization was documented successfully for varieties of crops like paddy (Evera, 2003), sorghum (Thangavel, 2003), groundnut (Vimal, 2003), lucerne (Kumar, 2003), mustard (Vijayageetha, 2007) and oats (Sumathi and Balamurugan, 2013).One another method is cluster analysis, a method used for grouping of genotypes showing similarities in one or more characters. For cultivar identification and discrimination, cluster analysis provides information on genetic background of parental population and genetic purity of next generations that clusters with the parents.

The results of the cluster analysis (Figure 1) revealed that these ten varieties can be grouped into two major clusters primarily based on Aspect Ratio. Those genotypes exhibiting Aspect Ratio values more than 2 were grouped as single major cluster which consists of four Tindivanam released varieties *viz.*, TMV 3, TMV 4, TMV 5, TMV 7 and one Coimbatore variety CO 1.The second cluster is also formed based on the Aspect Ratio values less than 2 which comprises of five varieties released from Tindivanam(TMV 6), Vriddhachalam (VRI 1, VRI 2 and VRI 3) and Srivilliputhur (SVPR 1).The sub-cluster 1 consists of two varieties (CO 1 and TMV 3) which shares common pedigree of TMV 3. Hence, these varieties were clustered into single sub-cluster. Similarly, in sub-cluster2, TMV4, TMV5 and TMV7 varieties were accommodated. All these varieties were developed through pure-line selection from Srivaikundam and Sattur local of southern districts of Tamil Nadu. Hence, these landraces may share common pedigree in the evolution. Hence, without any ambiguity these varieties were clustered in a single sub-cluster.

Further, circularity, rectangularity and perimeter values were also very similar in forming the sub clusters(1, 2) within major clusters (1). The major cluster2 comprised of five varieties which are mostly recorded less than 2 Aspect Ratio values. The sub-cluster 3 represents SVPR1 which is very unique with white coloured seed coat. VRI3 is also clustered adjacent toSVPR1 because SVPR 1 as one of the parent for evolution of VRI 3. Both, SVPR1 and VRI3 has similar circularity, rectangularity and perimeter. This forms the reason for forming nearby cluster. Sub-cluster 4 comprised of two varieties *viz*., TMV 6 and VRI 2. The variety VRI 2 has the pedigree from TMV6, hence formed a single sub-cluster. The findings denote that seed shape is governed by genetic mechanism and is directly inherited to its progenies. The seed shape plays a major role in grouping of varieties based on its Aspect Ratio, circularity and rectangularity.

The genotypes found in major cluster2 expressed similar values of circularity, rectangular and perimeter. The clustering pattern of above genotypes clearly indicated that variations present in the seed shape in between clusters and no variations or fewer variations were observed within the cluster. The clustering pattern based on seed morphological characters showed that the clusters consisted of genotypes from different geographical origin. In some cases, geographical origin had also a role in cluster composition. Similar findings were also reported by Tanabata *et al*. (2012).

The clustering pattern of the genotypes clearly indicates the discrimination of varieties can be done based on Aspect Ratio, circularity, rectangularity and perimeter parameters. In this study, CO 1 and TMV 3 are more diverse from TMV6 and VRI2 because it recorded higher and lower values of Aspect Ratio and were grouped in extreme clusters respectively. It is concluded that, morphological parameters could be used as the tool to discriminate the sesame varieties.

Hence, it was concluded that measuring the seed morphological characters using image vision system in sesame paved the way to know the pedigree of the variety apart from discriminating the varieties based on the physical properties.

Acknowledgement
We thank Dr. A.Yuvaraja, Assoc. Professor (PBG), Dept. of Plant Breeding and Genetics for his assistance for cluster analysis and comments that greatly improved the manuscript.

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Electronic Journal of Plant Breeding, 10 (2): 785-790 (Jun 2019) DOI: 10.5958/0975-928X.2019.00104.2
ISSN 0975-928X

Table 1. Measurement of seed morphological characters in sesame cultivars using image analysis

Variety	Area	Width	Length	Perimeter	Aspect ratio	Circularity	Rectangularity
CO 1	3.36 cd	1.57 de	3.16 bc	7.34 cd	2.03 ab	0.76 e	2.97 c
TMV 3	3.34 cd	1.54 e	3.15 bc	7.36 cd	2.06 a	0.77 e	2.98 bc
TMV 5	3.19 d	1.52 e	3.03 d	7.15 e	2.00 ab	0.78 de	2.90 b
TMV 4	3.27 cd	1.55 de	3.09 cd	7.27 de	2.01 ab	0.79 bc	2.94 b
TMV 7	3.31 cd	1.57 de	3.05 d	7.23 de	1.96 bc	0.79 b	2.92 b
SVPR 1	3.92 a	1.68 ab	3.32 a	7.88 a	1.98 bc	0.79 b	3.13 a
VRI 3	3.64 b	1.72 a	3.08 cd	7.52 bc	1.81 d	0.81 a	2.99 b
VRI 1	3.61 b	1.64 bc	3.19 b	7.59 b	1.96 bc	0.78 cd	3.03 b
TMV 6	3.42 c	1.60 cd	3.09 cd	7.38 cd	1.95 c	0.79 bc	2.96 b
VRI 2	3.41 c	1.59 cd	3.10 cd	7.35 cd	1.97 bc	0.79 bc	2.96 b
MEAN	**3.45**	**1.60**	**3.13**	**7.41**	**1.97**	**0.79**	**2.98**
SEd	**0.088**	**0.028**	**0.041**	**0.098**	**0.035**	**0.004**	**0.004**
CD (P=0.05)	**0.174**	**0.056**	**0.084**	**0.197**	**0.072**	**0.009**	**0.008**

DOI: 10.5958/0975-928X.2019.00104.2

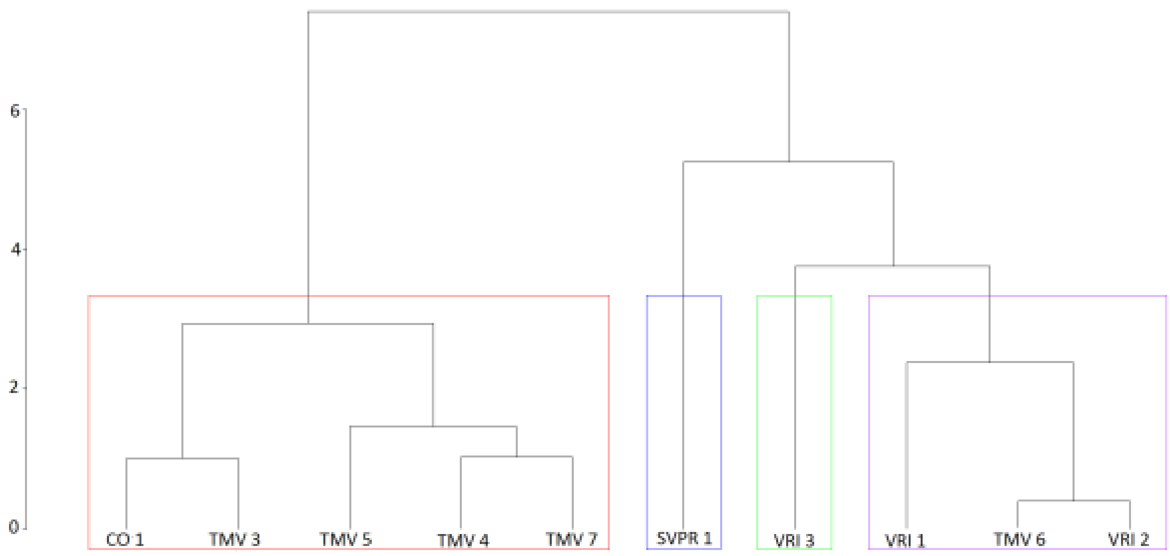

Fig. 1.Dendrogram showing cluster analysis for seed geometry using image analysis