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Characterization of Mustard Genotypes Through Image Analysis

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ABSTRACT

Varietal identification or discrimination of cultivars is essential for quality seed production. The measurement of geometry of single seed is possible with image analysis technique. Image analysis is an attractive system easily employed in many environments, non destructive, give a real-time analysis and inexpensive. Variation in seed morphology was observed for all the genotypes studied using image analyzer. The cluster analysis revealed that the genotypes could be grouped as two main clusters in which all genotypes except RN 393 formed one cluster and RN 393 as another cluster which showed that genotype in cluster one performed similarly for most of the geometric measurements of seed viz., area (2.82 cm²), perimeter (8.24 cm), radial variance (1.20) and circularity (1.15). Thus, morphological characterization through seed image analysis was found useful to discriminate the genotypes.

Key words: Seed geometry, clustering, image analyzer, machine vision, cultivar identity

INTRODUCTION

Image analysis technique (machine vision system) is one of such systems offers the prospect that researchers will be able to study seed surface features more closely and hence increase the available character set. Thus it has potential use in a wide range of tasks such as determining the cultivar identity of seed lots and testing of the distinctness of new cultivars for the award of breeders' right and cultivar registration (Keefe and Draper, 1986). Machine vision has been utilized for cultivar description, characterization and identification of varieties using seeds and plant parts (Draper and Travis, 1984; Van de Vooren *et al.*, 1991) measured pod length and width using image analyzer and compared with manual measurement in French bean. Image analysis system was used by Aquila *et al.* (2000) to measure area, perimeter and length of white cabbage seeds in order to monitor changes in seed physical parameters during imbibition and suggested that image analysis techniques have high potential in seed biology studies. The image analysis technique can be used to analyze other planktonic classes (Blackburn *et al.*, 1998). Morphometric analysis of digital images of germinated pollen found the greatest pollen tube area to pollen grain ratio with B and K medium + 30 mM sucrose (Wendy *et al.*, 2002). Image analysis has greatly simplified the measurement of root systems, allowing more detailed and accurate assessment of standard root variables (Carlos *et al.*, 2002). Sahoo *et al.* (2000) documented varietal discrimination of sunflower seeds using machine image approach. Anouar *et al.* (2001) grouped that four types of carrot seeds based on seed size using image analysis system. Shahin and Symons (2003) differentiated the lentil seeds based on change of colour, uniformity and discoloration due to developmental variation which could be traced through machine vision. Varietal characterization through image analysis

technique was successfully employed by Thangavel (2003) in sorghum; Kumar (2003) in Lucerne; Shete (2004) in castor; Suma (2005) in sesame and Venora *et al.* (2006) in phaseolus. The genetically pure mustard genotypes were obtained from National Research Centre on Rapeseed and Mustard, Bharatpur, Rajasthan. Keeping in view the above facts, the present study was initiated with the objective of characterization of mustard genotypes through image analysis. A computer imaging technique was developed for describing and classifying giant ragweed seeds using digital images of the seed top and side views (Sako *et al.*, 2001). Dana and Ivo (2008) stated that Computer image analysis to group together flax cultivars (*Linum usitatissimum* L.) according to their similarity in commercially important dry seed traits.

MATERIALS AND METHODS

The genetically pure mustard genotypes were obtained from National Research Centre on Rapeseed and Mustard, Bharatpur, Rajasthan. With a view to realize the objectives enumerated in the introduction chapter, the laboratory experiments were carried out in the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore between 2004 to 2007. The experimental details and methods adopted are enumerated hereunder.

Measurement of geometry of seed using image analysis technique: The genetically pure seeds of the above varieties of mustard were subjected to image analysis technique with three replication having twenty five seeds each. The methodology and the parameters studied are described below.

Image analysis system: Image analysis was carried out using Delta-T© (Delta-Instrument Device-Cambridge, UK) image analysis system by running custom written software win DIAS (Webb and Jekins, 2000).

For every replication twenty five seeds were placed on lighting hood in such a way that embryo axis of seed facing image analysis system and longitudinal axis of the seed running parallel to the surface of the camera lens. Seeds were viewed with video camera (DSP surveillance colour CCD camera CVS 200/3300) using transmitted light, so that a binary image of the silhouette of the seed was recorded by the 'WinDIAS'. The image of the support was removed by software after image grabbing in the computer that leaves an image of the object consist five rows and five columns for geometric data measurement.

Data measurement: Before going to actual measurement, calibration was done by placing transparent plastic rule on the lighting hood illuminated from below. Rule was aligned diagonally across the field of view and focus was adjusted to sharpen the image. Again, aperture adjustment was done until optimum colour and contrast was achieved. Input length was given in centimeter.

To measure description like area, perimeter, length and width from the menu object meter was selected. After setting the image was grabbed using image grabber and colour threshold was done until the entire area was highlighted. By logging the data, click the measurement bottom, the entire data were extracted by every time clicking entire objects. Data were viewed from the review and mean data for each parameter were summed up for average value in the win DIAS itself. The entire image and data were saved in the document file and interpreted data results were reported. The parameters studied are as follows:

- Area:** Multiplication of length and breadth of the object and expressed in cm²
Perimeter: Multiplication of length, breadth and height of the object and expressed in cm
Length: Distance between two points marked on screen using the mouse (or) diameter of the smallest circumscribed circle that will fit around an object and expressed in cm
Width: Length is measured in horizontal X-axis and expressed in cm
Shape factor: Shape factor is the ratio of the actual perimeter to that of a circle with the same area:

$$S = \frac{P}{P_C}$$

where, P is the perimeter of the object and P_C is the perimeter of a circle with the same area as the object. P_C is calculated as follows:

$$P_C = 2 (\pi \times A)^{0.5}$$

where, A is the actual area of the object.

Circularity: Circularity is the square root of the ratio of the actual area of the object to the area of a circle with the same circumscribed shell:

$$C = \sqrt{\frac{A}{AP}}$$

where, A is the actual area of the object, AP is the area of a circle with a diameter equal to the circumscribed diameter (or) length of the object.

Statistical analysis: The data collected for different parameters from the laboratory experiments were statistically analyzed by the 'F' test for significance as suggested by Panse and Sukhatme (1985). The critical difference CD was computed at 5 per cent probability. Where ever necessary, the per cent values were first transferred to angular (arc sine) value before analysis.

Correlation analysis: The correlation analysis was done using SPSS 75 package (www.spssinc.com).

RESULTS AND DISCUSSION

The individual seed area was recorded maximum in Kranti (4.19 m²) and it was minimum in Maya (1.93 cm²). Maximum perimeter of the seed was measured in Maya (10.8 cm), whereas it was minimum (6.94 cm) in Rohini. Perimeter was observed highest in Bio 902 (10.8) and lowest in genotype Maya (6.44). The length of the seed was maximum (2.45 cm) in Bio 902 and it was minimum (1.80 cm) in Maya. The genotype Bio 902 expressed maximum (2.15 cm) width while it was minimum (1.50 cm) in Maya. Genotype Bio 902 registered the maximum shape factor of 1.63 while it was minimum in Kranti (1.02). The circularity of seed was recorded highest (1.37) in GM-2 and lowest in Varuna and Pusa Bold (0.84) (Table 1). In cluster analysis two major clusters were

Table 1: Seed morphological characters measurement of mustard cultivars using image analysis system

Parameters		Cultivars								
		Rohini	BIO 902	Kranti	Maya	GM-2	Varuna	Per 7	Pusa Bold	RN 393
Area (cm ²)	(1) Mean	2.19	3.58	4.19	1.93	3.56	2.52	2.25	2.48	2.82
	(2) SED	1.08	1.01	0.92	0.79	1.21	1.00	1.21	0.732	0.658
	(3) Co. eff. Mean	49.50	28.10	22.00	40.70	33.90	39.80	53.90	29.50	23.30
Perimeter (cm)	1	6.94	10.80	7.34	6.44	8.88	7.92	7.48	9.10	8.24
	2	2.76	2.54	0.88	2.54	4.27	3.04	4.05	2.99	2.75
	3	39.80	23.50	11.90	39.40	48.10	38.40	54.20	32.80	33.40
Length (cm)	1	1.83	2.45	2.41	1.80	2.12	2.15	1.85	2.13	2.06
	2	0.54	0.29	0.253	0.55	0.90	0.60	0.81	0.47	0.58
	3	29.30	11.70	10.50	30.40	42.50	28.10	44.00	22.00	28.30
Width (cm)	1	1.57	2.15	2.10	1.50	1.86	1.71	1.57	1.86	1.80
	2	0.49	0.35	0.28	0.48	0.78	0.42	0.72	0.41	0.51
	3	31.40	16.40	13.50	32.10	42.00	24.70	46.20	21.80	28.00
Shape factor	1	1.32	1.63	1.02	1.28	1.34	1.39	1.38	1.61	1.41
	2	0.26	0.34	0.01	0.27	0.54	0.36	0.49	0.36	0.46
	3	19.70	20.90	1.10	20.70	40.50	25.60	35.20	22.60	32.20
Circularity	1	0.91	0.86	0.95	0.88	1.37	0.84	1.06	0.841	1.15
	2	0.12	0.04	0.03	0.11	1.16	0.10	0.62	0.08	0.95
	3	12.90	4.50	3.28	12.10	85.00	11.50	58.30	10.00	82.30

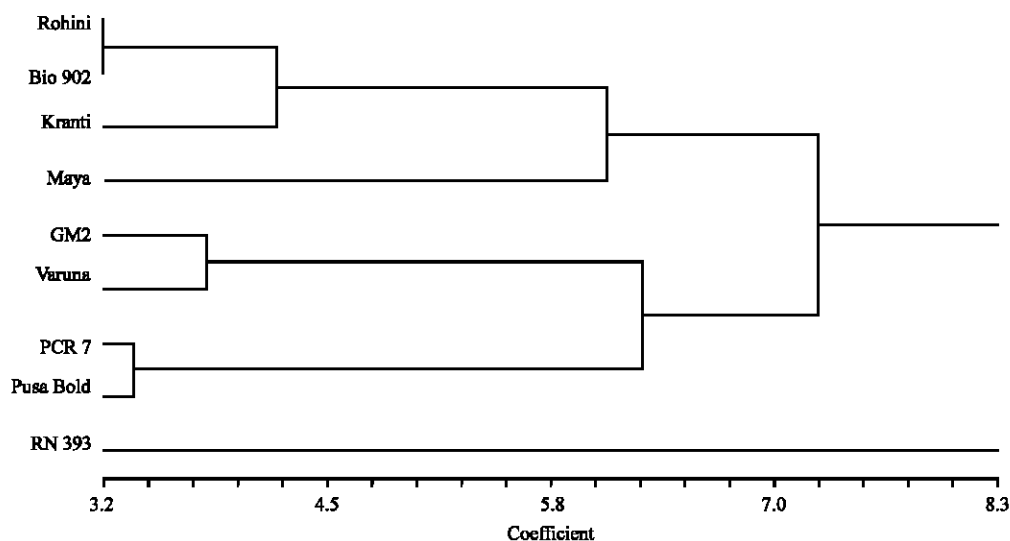


Fig. 1: Hierarchical cluster analysis for geometry of seed using image analysis technique

found in respect to the measurement of geometry of seed by image analysis technique. The genotype RN 393 formed as one major cluster while the other mustard genotypes belonged to the second major cluster (Fig. 1).

The measurement of geometry of single seed is possible with image analysis technique. Image analysis is an attractive system easily employed in many environments, non destructive, give a real-time analysis and inexpensive (Myers and Edsall, 1989). Mudzana *et al.* (1995) compared manual evaluation with image analysis system for plant morphology. Dehghan-Shoar *et al.* (1998)

discriminated several varieties of Lucerne by capturing images of seed morphological characters and recommended this method for seed certification. Image analysis system was used by Aquila *et al.* (2000) to measure area, perimeter and length of white cabbage seeds in order to monitor changes in seed physical parameters during imbibitions and suggested that image analysis techniques have high potential in seed biology studies. Anouar *et al.* (2001) grouped that four types of carrot seeds based on seed size using image analysis system. Shahin and Symons (2003) differentiated the lentil seeds based on change of colour, uniformity and discoloration due to developmental variation which could be traced through machine vision. The thickness of the cuticle, size and density of cells and cell walls of apple were studied using Image analyzer to better understanding the differences in texture and firmness or crispness among the three varieties of apples are examined as Braeburn, Fuji and the Golden Delicious (Ouattara *et al.*, 2011). The shape coefficient for mineral grains of sieve sizes below 0.3 mm can be calculated using image analyzer (Peszko *et al.*, 2007). A karyological analysis on three naturally growing *Limonium miller* species were made using image analysis system. The species studied are; *Limonium iconicum* (Boiss. and Heldr.) O. Kuntze, *L. lilacinum* (Boiss. and Bal.) Wagenitz and *L. globuliferum* (Boiss. and Heldr.) O. Kuntze. The chromosome numbers are determined as $2n = 34$ in *L. iconicum*, as $2n = 36$, in *L. lilacinum* and as $2n = 18$ in *L. globuliferum*. The chromosome lengths in these taxa varied between 1.44 to 6.10 μm . The karyotype formula of the species studied were; in *L. iconicum* $10m + 5sm + 2T$, in *L. lilacinum* $7m + 10sm + 1T$ and in *L. globuliferum* $4m + 5sm$ (Evliyaoglu *et al.*, 2008). Image analysis was performed using Image master 2D Platinum 5.0 to determine proteins regulated by water stress in Peanut variety Khon Kaen 4, a water-stress sensitive variety (Akkasaeng *et al.*, 2007). The seed morphology of 11 species of *Silene* was studied, utilizing light and scanning electron microscopes, to determine the significance of seed coat features as taxonomic characters. Seed shapes are reniform or reniform-circular. The colour of seeds is greenish or brown. Seed size varies from the 0.5-1.2 mm long. The lateral surface is flat or concave. The dorsal surface is concave grooved in all examined taxa except *S. longiflora* is convex. The periclinal walls are convex, granulate with tubercle in the central area; convex, granulate; flat, granulate or flat, smooth. The anticlinal walls are straight, S-undulate, U-undulate or V-undulate (Fawzi *et al.*, 2010). Seed morphological characters and seed coat sculpture of 14 species of Malvaceae were examined to assess systematic implications of seed coat sculpture (El-Naggar, 2001). The characteristics of the seeds of 7 annual and perennial species of *Trifolium*, *Lotoidea* section, in Iran were investigated by means of Scanning Electron Microscopy (SEM) and a stereomicroscope (Salimpour *et al.*, 2007). The flowers, leaves, fruits, seeds and pollens of *R. communis* from different phytogeographical regions were examined with both scanning electron and light microscopy. Twenty two morphological characters were defined by Shaheen (2002).

The genotypes Rohini, Boi 902, Kranti, Maya, GM-2, Varuna, PCR 7 and Pusa Bold were closely grouped under one cluster (Fig. 1) due to close linkage of seed characters while, RN 393 formed a separate cluster. It was revealed that, the conventional way of grouping the cultivars based on seed character was also developed same clustering pattern with some minor variations. Similar results were obtained by Kumar (2003) in lucerne (Shete, 2004) in castor (Suma, 2005) in sesame (Nisha, 2007) in wheat and (Sumathi, 2007) in oats.

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