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Establishing distinctiveness in phenotypically similar rice (*Oryza sativa* L.) varieties using image analysis

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Abstract

India is one of the centers of origin for rice, where large number of native varieties and land races are available and grown in different agro-climatic zones having unique characteristics and great adaptability. Variety identification is of prime importance under the PPV&FR Act, rice varieties are registered on the basis of Novelty, Distinctiveness, Uniformity and Stability (NDUS) characters which are taken as per the stage of observations listed in DUS guidelines. The scope of morphological differences between the varieties is less due to narrow genetic base, and requires skilled human power which is subjective in nature. Also this process is time, labour and cost intensive. Hence new techniques are needed, thus the present study was proposed with the objectives of establishing distinctiveness in phenotypically similar rice (*Oryza sativa* L.) varieties using image analysis. The study grouped the 28 varieties into five different clusters based on these DUS parameters. An exhaustive image library for various plant parts viz. seed, leaf, stem and panicle was also developed for the material under study. Two different types of softwares were used for extraction of features from the images viz. Grain Analysis Software (for size and shape features) and MATLAB software (for textural features). The softwares for extracting features from stem and panicle were still under construction by the end of study, hence the stem and panicle images were not used for feature extraction. Based on the comparative clustering patterns on four different kinds of features viz. DUS parameters, seed imaging, leaf imaging and DUS in combination with imaging; the study revealed that image features extracted from seed were most helpful for distinguishing the varieties.

Keywords: MATLAB software, Machine vision, variety differentiation etc.

Introduction

Cultivated rice (*Oryza sativa* L.) belongs to family Poaceae and, at present sustains two thirds of the world's population. India is one of the centers of origin for rice, where large number of native varieties and land races are available and grown in different agro-climatic zones having unique characteristics and great adaptability. Variety development is time consuming process; therefore breeder of the variety should get some reward for that such effort in terms of royalty and protection. Hence, India has developed its *sui generis* system for Plant Variety Protection of new varieties for granting Plant Breeder's Rights (PBR) to the breeder/farmers/Institution known as Protection of Plant Variety & Farmer's Right Act 2001 (PPV&FRA).

There are about 950 released and notified varieties of rice (*Oryza sativa* L.) in India for which diagnostic features are well known and the same are followed for the purpose of seed certification. Hence, variety identification is of prime importance. However, under the PPV&FR Act, rice varieties are registered on the basis of Novelty, Distinctiveness, Uniformity and Stability (NDUS) characters which are taken as per the stage of observations listed in DUS guidelines (Chakrabarty *et al.* 2012) ^[1]. The scope of morphological differences between the varieties is less due to narrow genetic base, and requires skilled human power which is subjective in nature. Also this process is time, labour and cost intensive. Hence, the manual identification of varieties/seeds by specialized technicians is slow, has low reproducibility, and possesses a degree of subjectivity that is hard to quantify. Image analysis is a promising technique which can contribute significantly in improving the variety identification since it replaces the laborious manual inspection with cameras and image processing operations. Digital image analysis offers an objective and quantitative method for estimation of morphological parameters. Kaur and Verma (2013) ^[2] proposed computer vision technique for grading of rice kernels based on their sizes (full, medium, half). Singh and Banga (2012) ^[3] proposed image processing techniques for grading of rice samples based on their sizes.

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Therefore, since DUS Testing procedure is time consuming and subjective; and establishing distinctiveness among phenotypically identical rice varieties on the basis of morphological parameters alone is difficult; hence new techniques are needed, thus the present study was proposed with the objectives of establishing distinctiveness in phenotypically similar rice (*Oryza sativa* L.) varieties using image analysis.

Materials and Methods

The field experiment was conducted during *kharif* season of 2014-15 in the field of Seed Science and Technology Division and Genetics Division, IARI, New Delhi. The image analysis work was done as per the designed protocol in laboratory of Division of Seed Science and Technology, IARI, New Delhi and at Central Institute of Agricultural Engineering, Bhopal. The complete DUS database of plant morphological characters (27) for all the rice varieties were generated as per DUS guidelines (Rice) no. SG/ 01/ 2007.

Type of assessment followed for 27 characteristics as under

MG: Measurement by a single observation of a group of plants or parts of plants

MS: Measurement of a number of individual plants or parts of plants

VG: Visual assessment by a single observation of a group of plants or parts of plants

VS: Visual assessment by observation of individual plant or parts of plants

Imaging Protocol

The imaging of plant parts (leaf and seed) was done by the following ways:

Seed	Scanner 1. Canon LiDE 110 2. Resolution: 600 Dpi 3. Document size: Platen
Leaf	Photography set up 1. Height of Camera: 18cm 2. Lens focus setting: between 0.3m-0.5m 3. Lighting type: Back light with standard reflector 4. Light power setting: 1.0 5. Distance of light source: 20cm below as per size 6. Exposure parameter: F=20,ISO=100, Shutter speed = 60, White balance = Auto flesh 7. Gadget used for holding sample: Cartridge sheet

Image Processing and extraction of features

The processing of images was done at Seed Science and Technology Division, IARI New Delhi and CIAE, Bhopal MP. The image processing was done by using two different

kinds of softwares:

- Grain Analysis Software developed by Dr. Nachiket Kotwaliwale.
 - a. Used for extraction of size and shape features from the seed.
- MATLAB software (version 7.12.0.635,R2011a) developed by Dr. Nachiket.
 - a. Used for extraction of textural features from the seed and leaf images.

Eleven parameters are measured from the Grain Analysis Software: Area, Perimeter, Bounding box length, Bounding box width, Axial length, Axial width, Median length, Median width, Eccentricity, Roundness, Equivalent diameter.

The basic features recorded by the MATLAB software

1. **Morphological features:** Length, Width, Awn length, Kernel area, Kernel perimeter, Major axis, Minor axis, Eccentricity, Equivalent Diameter, Length-width ratio
2. **Textural features:** Contrast; Correlation; Energy; Homogeneity; Range; STD; Entropy; Offset 0; Offset 45; Offset 90; Offset 135; SRE; LRE; GLN; LP; RLN; LGRE; HGRE
3. **Chromatic features:** Redness; Greenness; Blueness; Hue; Saturation; Value; Hue Std; RHS colour value

Result and Discussion

A total of three grouping characters and twenty four essential characters as per the DUS guidelines (Rice) no. SG / 01 / 2007; were recorded for the research material at various growth stages starting from basal leaf sheath colour (at seedling stage) upto grain length and width (after harvesting). Out of twenty seven morphological descriptors studied, three were found monomorphic, two were dimorphic and rests twenty two were found polymorphic in state of expression. Therefore, the following seven important quantitative DUS traits were used for grouping the varieties:

1. Leaf: Length of blade
2. Leaf: Width of blade
3. Stem: Length excluding panicle
4. Panicle: Length of main axis
5. Grain length
6. Grain width
7. Time of heading: Since this trait is one of the important traits used universally for grouping of varieties; hence used for clustering.

The dendrogram formed by using the above traits is depicted in Fig. 1 and the corresponding clustering pattern is shown in Table 1.

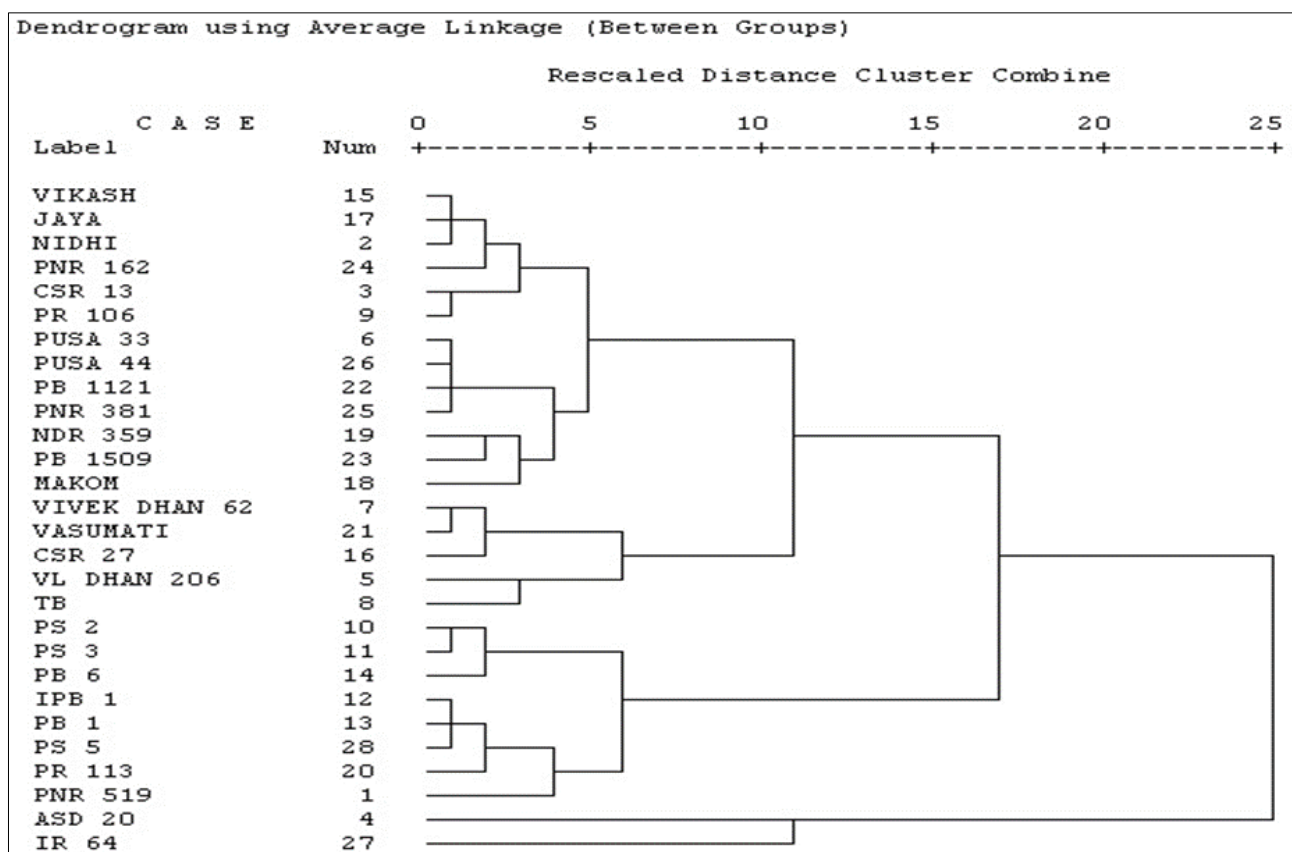


Fig 1: Dendrogram based on DUS parameters

Table 1: Clustering pattern of varieties based on DUS parameters

Cluster	Number of Varieties	Name
I	06	VIKASH, JAYA, NIDHI, PNR-162, CSR-13, PR-106
II	07	PUSA-33, PUSA-44, PB-1121, PNR-381, NDR-359, PB-1509, MAKOM
III	05	VIVEKDHAN-62, VASUMATI, CSR-27, V L DHAN-206, TARAORI BASMATI
IV	08	PS-2, PS-3, PS-5, PB-6, IMPROVED PB-1, PB-1, PR-113, PNR-519
V	02	ASD-20, IR-64

Table 2: Seed data generated by Grain analysis software

S. No	VAR	Area, mm ²	Length, mm	Breadth, mm	Eccentricity	Perimeter, mm	Equivalent Dia, mm	Roundness	Axial Length, mm	Axial Width, mm	Median Length, mm	Median Width, mm
1	CSR-13	13.421	8.74	2.1	0.973	19.66	4.13	0.2227	4.8	1.98	3.5221	1.66
2	Imp PB-1	19.912	11.93	2.49	0.98	26.4	5.04	0.1782	6.35	2.26	5.0165	1.9
3	Nidhi	14.278	10.1	2.06	0.979	22.3	4.26	0.1791	5.03	1.83	4.0131	1.56
4	PB-1	17.636	11.55	2.17	0.982	25.44	4.73	0.1678	7.18	1.97	4.8301	1.66
5	Vivek dhan-62	15.768	7.71	2.79	0.931	18.07	4.48	0.3386	3.94	2.64	2.7646	2.31
6	VL dhan-206	17.09	8.74	2.69	0.948	20.01	4.66	0.2867	3.74	2.58	2.6946	2.19
7	IR-64	15.538	9.41	2.33	0.97	21.16	4.42	0.223	7.37	2.09	3.9475	1.81
8	PB-1121	21.639	12.65	2.39	0.985	27.6	5.25	0.1719	5.61	2.31	3.2006	1.85
9	PB-1509	21.783	13.25	2.31	0.984	28.76	5.24	0.1592	7.82	2.23	4.6059	1.88
10	PNR-162	13.658	8.92	2.16	0.971	19.97	4.18	0.2199	4.32	2	3.086	1.66
11	PNR-381	14.776	9.36	2.13	0.975	20.86	4.34	0.2156	4.13	2.04	3.1412	1.7
12	PUSA-44	16.009	9.05	2.34	0.967	20.58	4.5	0.249	5.59	2.22	4.1274	1.93
13	ASD-20	16.574	9.82	2.37	0.973	22.12	4.6	0.2197	5.63	2.12	4.6057	1.85
14	JAYA	17.848	8.42	2.91	0.937	19.68	4.75	0.3214	4.12	2.8	2.9294	2.38
15	PNR-519	16.108	9.33	2.32	0.968	21.13	4.52	0.236	7.36	2.17	4.6354	1.94
16	PR-106	16.64	9.15	2.53	0.961	20.87	4.6	0.2544	6.28	2.36	3.884	2
17	PR-113	14.678	10.6	2.09	0.981	23.43	4.3	0.1663	6.49	1.86	5.4567	1.51
18	PS-2	22.663	12.38	2.61	0.978	27.53	5.37	0.1896	10.05	2.37	5.3637	2.08
19	PS-3	21.907	12.9	2.52	0.984	28.52	5.26	0.1679	9.66	2.23	5.9139	1.92
20	PS-1	20.841	11.75	2.41	0.977	25.96	5.15	0.1928	6.39	2.23	5.2747	2.01
21	T.BASMATI	17.584	11.88	2.42	0.981	26.33	4.72	0.1587	8.77	2.13	4.119	1.67
22	VASUMATI	20.312	9.29	2.9	0.949	21.53	5.08	0.2989	5.31	2.77	3.7762	2.42

23	VIKASH	15.836	10.31	2.31	0.975	22.89	4.48	0.1893	5.6	2.05	4.2122	1.71
24	CSR-13	17.601	9.58	2.47	0.966	21.69	4.73	0.2436	5.63	2.32	4.5847	2.02
25	MAKOM	18.23	8.52	2.84	0.94	19.91	4.81	0.3202	4.66	2.72	3.3974	2.39
26	NDR-359	19.732	9.54	2.78	0.955	21.88	5.01	0.2769	4.68	2.65	3.6577	2.28
27	PB-6	17.464	10.13	2.34	0.974	22.9	4.71	0.216	6.66	2.2	5.1096	1.89
28	PUSA-33	17.173	10.2	2.35	0.974	22.67	4.68	0.2112	5.29	2.18	3.446	1.85

Table 3: Validation of Grain analysis software generated data with manually recorded observation

	Bounding box length	Axial length	Median length	Length (Manual)	Bounding box width	Axial width	Median width	Width (Manual)
Bounding box length	-	-	-	-	-	-	-	-
Axial length	0.312	-	-	-	-	-	-	-
Median length	0.816*	0.362	-	-	-	-	-	-
Length(Manual)	0.912**	0.271	0.828*	-	-	-	-	-
Bounding box width	-	-	-	-	-	-	-	-
Axial width	-	-	-	-	0.693*	-	-	-
Median width	-	-	-	-	0.394	0.375	-	-
Width(Manual)	-	-	-	-	0.746*	0.211	0.497*	-

** . Correlation is significant at the 0.01 level (2-tailed)

* . Correlation is significant at the 0.05 level (2-tailed)

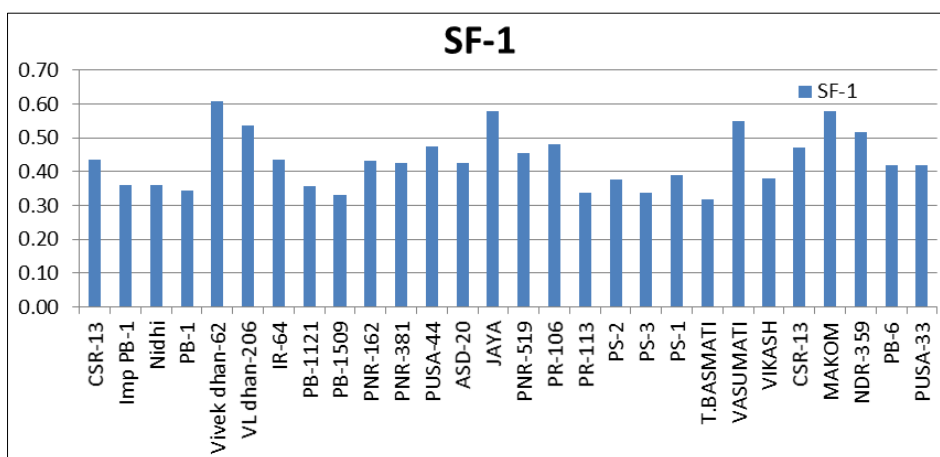
Validation of software generated parameters

The software generated three different kinds of length and width, viz. bounding box, axial and median. However, this is a dilemma for the user to find which of the three correspond best to the manually recorded observations. Hence, correlation was worked between the manually recorded grain length and width with the three type of measurements generated by the software. The perusal of the data revealed that the values of bounding box length and bounding box width were closest to the manual readings. Table 3 shows the

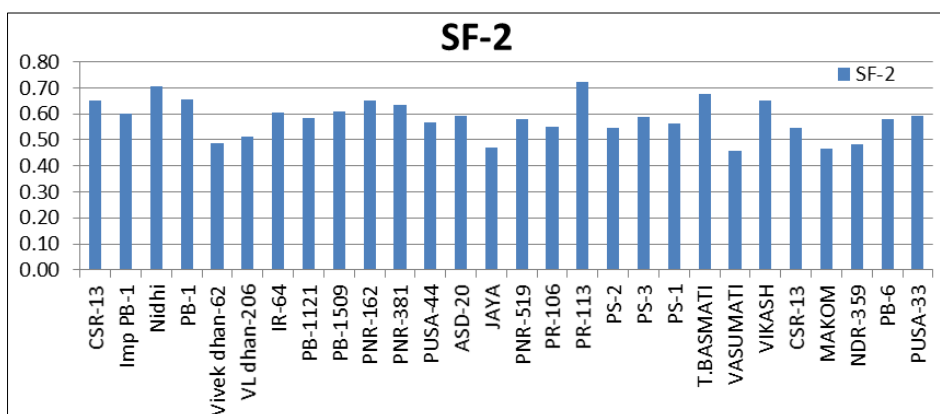
coefficient of correlation matrices for dimensions and shape factors of rice. Among the various length and width parameters computed from the software, the bounding box length and bounding box width were found to have more implication for varietal differentiation.

Shape Factors based on Feature extraction of Grains

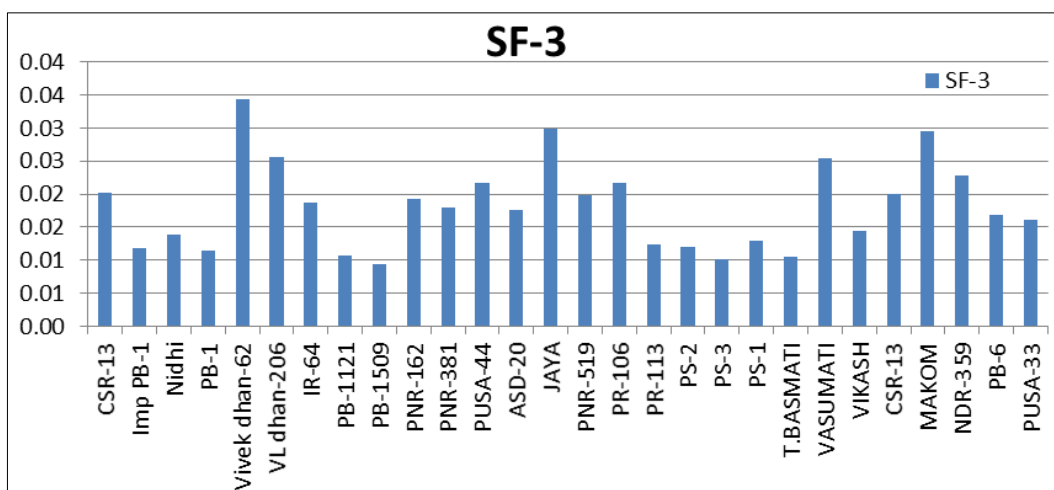
From the values of axis length, perimeter and area, shape factors were derived, following Symons and Fulcher (1988) formulae.



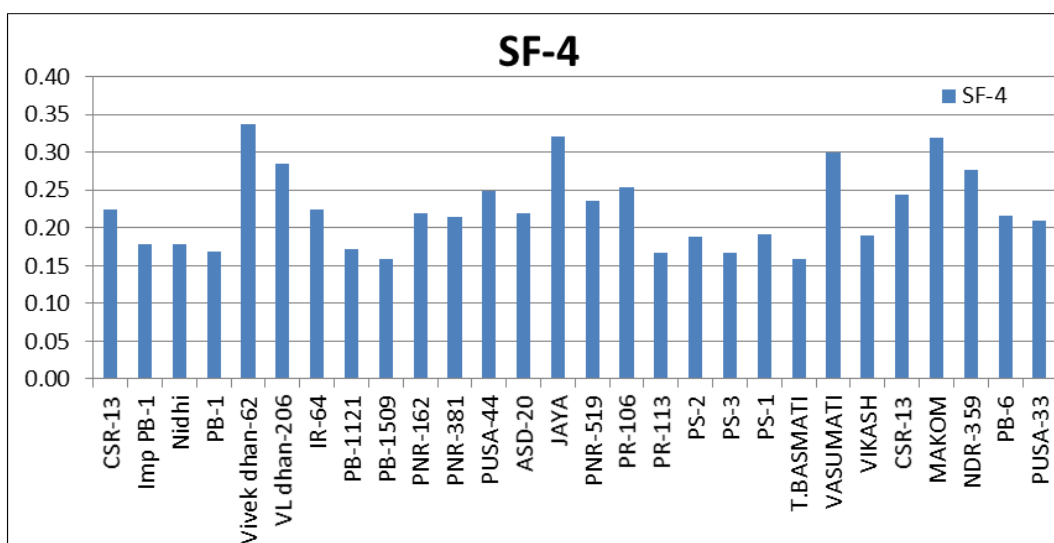
Shape factor 1: $4 \pi \text{ Area} / \text{Perimeter}^2$



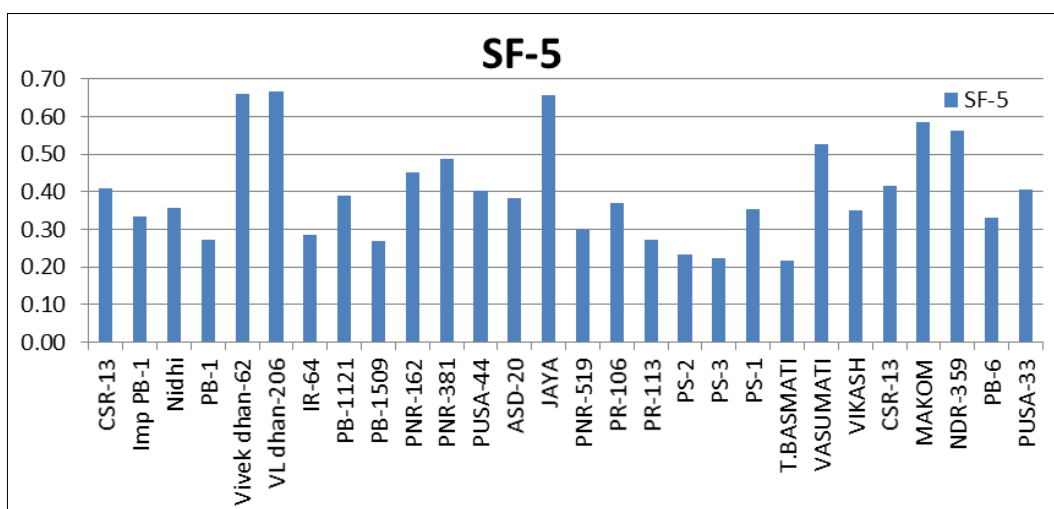
Shape factor 2: Major axis length/Area



Shape factor 3: Area/Major axis length³



Shape factor 4: Area/(Major axis length/2)(Major axis length/2)



Shape factor 5: Area/(Major axis length/2)(Minor axis length/2)

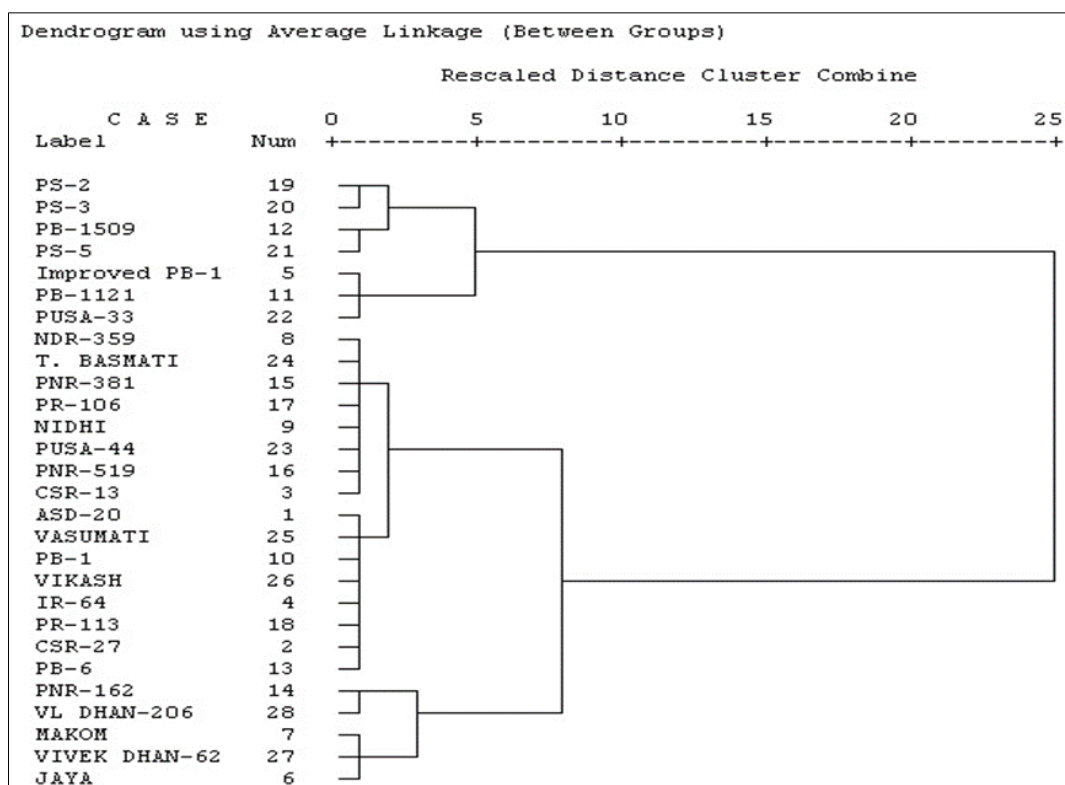


Fig 2: Dendrogram generation based on Seed data generated through MATLAB software

Table 4: Clustering pattern of varieties based on Seed data generated through MATLAB software

Cluster	Number of varieties	Variety Name
I	4	PS-2, PS-3, PB-1509, PS-5,
II	3	IMPROVED PB-1, PB-1121, PUSA-33
III	8	NDR-359, TARAORI BASMATI, PNR-381, PR-106, NIDHI, PUSA-44, PNR-519, CSR-13
IV	8	ASD-20, VASUMATI, PB-1, VIKASH, IR-64, PR-113, CSR-27, PB-6
V	2	PNR-162, VL DHAN-206,
VI	3	MAKOM, VIVEK DHAN-62, JAYA

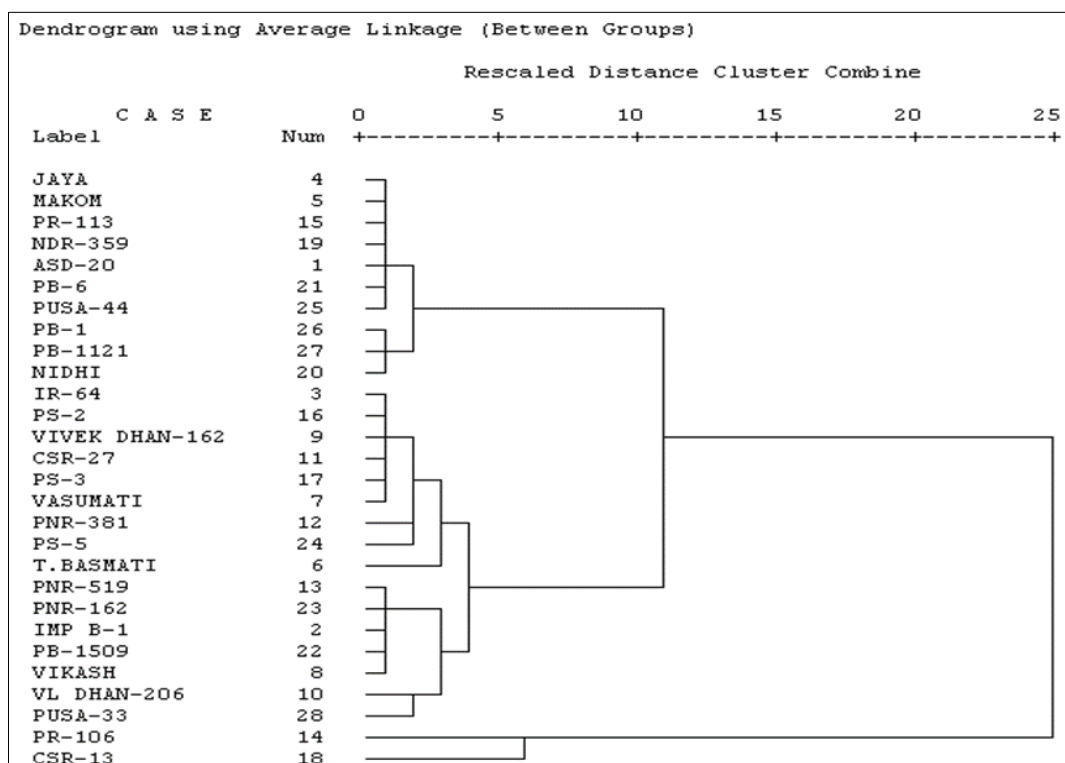


Fig 3: Dendrogram generation based on Leaves data

Table 5: Clustering pattern of varieties based on Leaf data

Cluster	Number of varieties	Variety Name
I	7	JAYA, MAKOM PR-113, NDR-359, ASD-20, PB-6, PUSA-44
II	3	PB-1, PB-1121, NIDHI
III	6	IR-64, PS-2, VIVEK DHAN-62, CSR-27, PS-3, VASUMATI
IV	3	PNR-381, PS-5, TARAORI BASMATI
V	5	PNR-519, PNR-162, IMPROVED PB-1, PB-1509, VIKASH,
VI	2	VLDHAN-206, PUSA-33
VII	2	CSR-13, PR-106

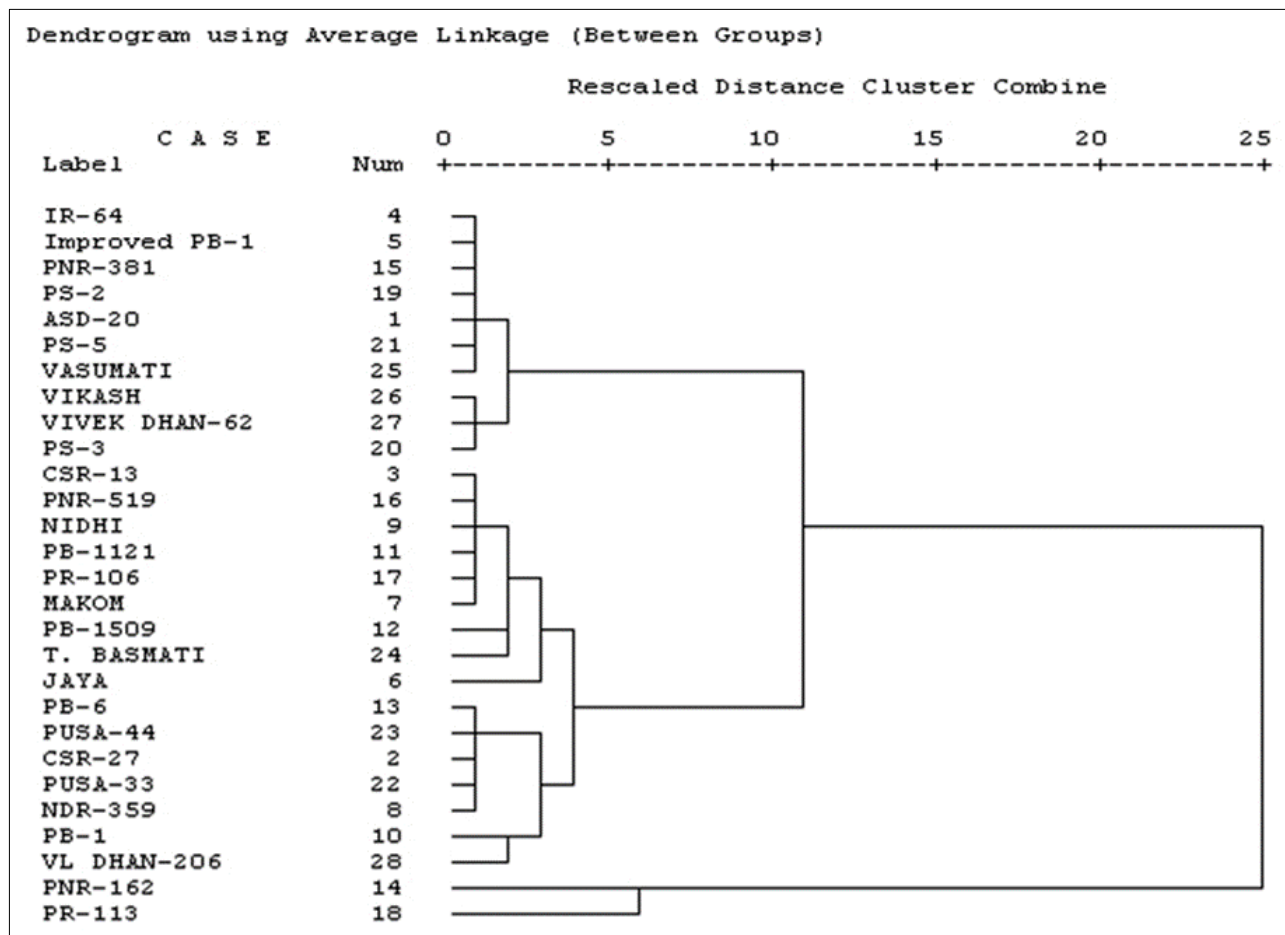


Fig 4: Dendrogram generation based on Seed and Leaves data

Table 6: Clustering pattern of varieties based on Seed and Leaves data

Cluster	Number of varieties	Variety Name
I	7	IR-64, IMPROVED PB-1, PNR-381, PS-2, ASD-20, PS-5, VASUMATI,
II	3	VIKASH, VIVEK DHAN-62, PS-3
III	6	CSR-13, PNR-519, NIDHI, PB-1121, PR-106, MAKOM
IV	3	PB-1509, TARAORI BASMATI, JAYA
V	5	PB-6, PUSA-44, CSR-27, PUSA-33, NDR-359,
VI	2	PB-1, VLDHAN-206
VII	2	PR-113, PNR-162

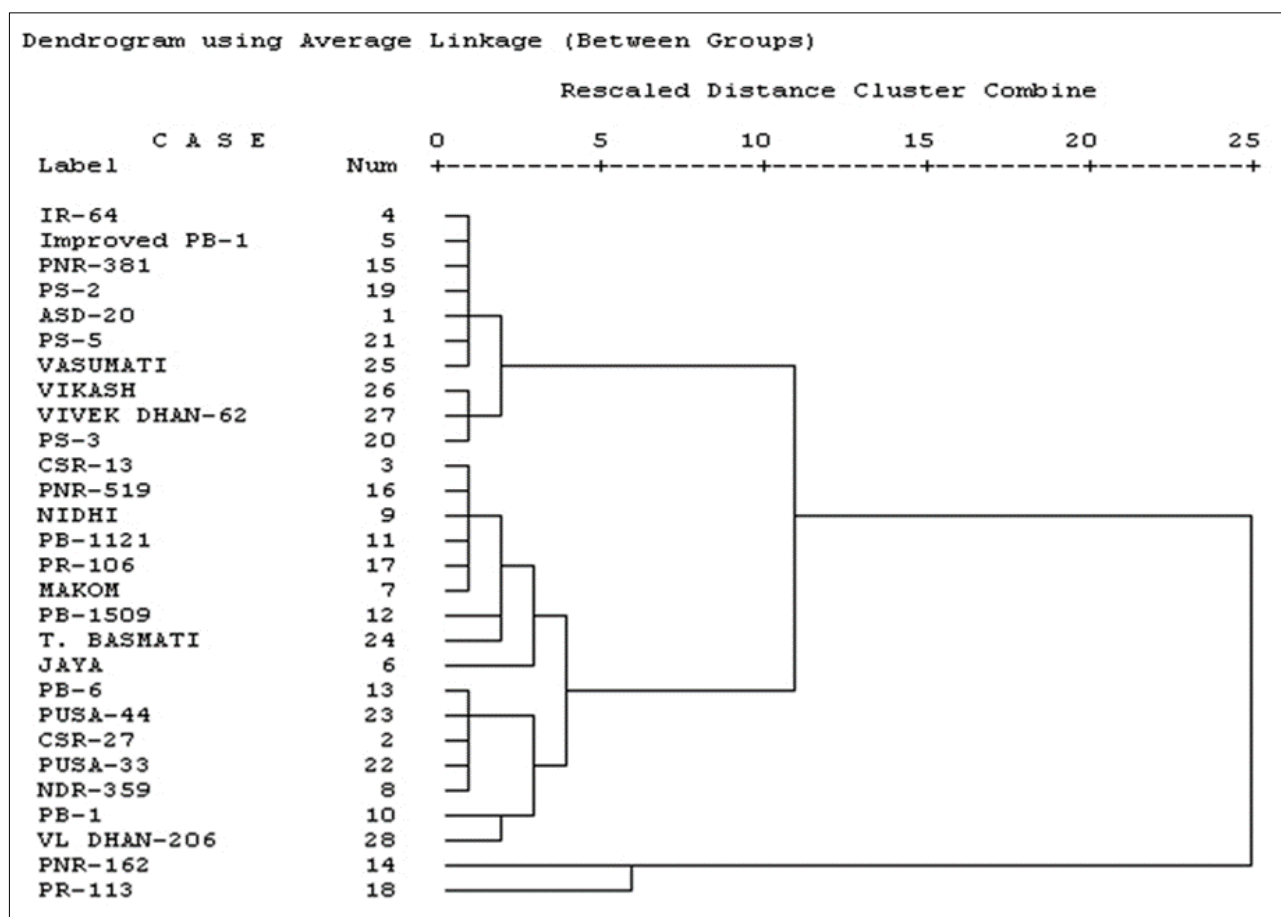


Fig 5: Dendrogram based on DUS parameters + Image features

Table 7: Clustering pattern of varieties based on DUS parameters + Image features

Cluster	Number of varieties	Variety Name
I	7	IR-64, IMPROVED PB-1, PNR-381, PS-2, ASD-20, PS-5, VASUMATI,
II	3	VIKASH, VIVEK DHAN-62, PS-3
III	6	CSR-13, PNR-519, NIDHI, PB-1121, PR-106, MAKOM
IV	3	PB-1509, TARAORI BASMATI, JAYA
V	5	PB-6, PUSA-44, CSR-27, PUSA-33, NDR-359,
VI	2	PB-1, VLDHAN-206
VII	2	PR-113, PNR-162

A perusal of the various shape factors revealed that varieties T. Basmati and Vivek Dhan 62 were significantly different from each other for Shape factor 1, varieties Vasumati and PR 113 for Shape factor 2, varieties PB 1509 and Vivek Dhan 62 for Shape factors 3 and 4, and T. Basmati and Jaya were significantly different from each other for Shape factor 5. A comparative assessment of various shape factors revealed that shape factor 5 involving area and major and minor axis length was most useful for distinguishing varieties.

The varieties of the present study were singled out using a combination of grouping characters and essential characters as per the DUS guidelines (Rice) no. SG/01/2007. Since the main objective of the study was to resolve differentiation using image analysis, hence the selection criteria for forming closely related groups included those plant parts which were used for imaging; viz. seed (length and width), leaf (length and width), stem (length), panicle (length) in addition to time of heading; since this trait is one of the important traits used universally for grouping of varieties; hence used for clustering. The study grouped the 28 varieties into five different clusters based on these DUS parameters.

Ranking of image features

Based on the comparative clustering patterns on four different kinds of features viz. DUS parameters, seed imaging, leaf imaging and DUS in combination with imaging; the study revealed that image features extracted from seed were most helpful for distinguishing the varieties. The size and shape features extracted by the Grain Analysis software as well as derived shape factors clearly distinguished the varieties into various clusters. The additional textural features extracted from seed images (about 27 textural features) further aided in the differentiation of varieties. This was followed by the image features extracted from the leaf images. The MATLAB software again extracted 27 textural features from both flag leaf and penultimate leaf; and from both sides. Since the softwares for extracting features from stem and panicle were still under construction by the end of study, hence the stem and panicle images were not of much significance during the course of study.

Therefore, the overall ranking of image features for the material of the present study is Seed > Leaf > stem/panicle. Zhu, F., et al. (2021) [4] developed an open-source graphical

user interface (GUI) software, Seed Extractor that determines seed size and shape (including area, perimeter, length, width, circularity, and centroid), and seed color with capability to process a large number of images in a time-efficient manner and identified known loci for regulating seed length (GS3) and width (qSW5/GW5) in rice, which demonstrates the accuracy of this application to extract seed phenotypes and accelerate trait discovery.

Ropelewska, E., & Rutkowski, K. P. (2021) ^[5]. Evaluated the usefulness of individual parts of fruit (skin, flesh, stone and seed) for cultivar discrimination of peaches based on textures determined using image analysis. Discriminant analysis was performed using the classifiers of Bayes net, logistic, SMO, multi-class classifier and random forest based on a set of combined textures selected from all color channels R, G, B, L, a, b, X, Y, Z and for textures selected separately for RGB, Lab and XYZ color spaces. In the case of sets of textures selected from all color channels (R, G, B, L, a, b, X, Y, Z), the accuracy of 100% was observed for flesh, stones and seeds for selected classifiers.

Seed morphological features obtained by processing radiographs with the Tomato Analyzer software and of red–green–blue obtained and processed on the Groundeye device were used to test differentiation of materials of the *Urochloa* genus. Seeds of *Urochloa brizantha*, *Urochloa ruziziensis* and *Urochloa decumbens* were evaluated. Morphological features obtained by Tomato Analyzer allowed differentiation of *Urochloa* seeds at an accuracy level greater than 80% for all the materials evaluated De Freitas *et al.* (2021) ^[6].

To develop discriminative models based on geometric features to distinguish seeds belonging to different apple cultivars, images of seeds of apples ‘Gala’, ‘Jonagold’ and ‘Idared’ were acquired using a flatbed scanner. In the case of models build based on selected linear dimensions, the accuracy of discrimination was equal up to 84% for distinguishing seeds of all three apple cultivars for the J48 classifier from Decision Trees and 93% for analysis of ‘Gala’ and ‘Idared’ for the J48 from Decision Trees. (Ropelewska, E., & Rutkowski, K. P. 2021) ^[7].

Vale, A *et al.* (2020) presented. Open source plugin for the automatic segmentation of an image of a seed sample. The new plugin was tested on a total of 3,386 seed samples from 120 species belonging to the Fabaceae family. Digital images were acquired using a flatbed scanner. The results showed that the new plugin was able to segment all of the digital images without generating any object detection errors. In addition, the new plugin was able to segment images within an average of 0.02s, while the average time for execution with the manual method was 63s. Sarigu, M *et al.* (2019) ^[9] studied 124 morpho colorimetric quantitative and qualitative features of seeds of the *Paonia mascula* group from the Balearic Islands, Corsica, Sicily, and Sardinia were measured by an image analysis system to evaluate whether differences in seed morphology and results showed that the seeds of the studied taxa were distinguishable with a high percentage of classification and highlighted that three different taxa are identifiable in Corsica (*P. corsica*, *P. mascula* subsp. *mascula*, and *P. morisii*), while only one (*P. cambessedesii*) is identifiable in the Balearic Islands.

Pacifico, L *et al.* (2019) ^[10] develop a new medicinal plant data set based on the extraction of texture and color features from plant leaf images. A complete automatic plant recognition system is proposed, and five well-known machine

learning classifiers are tested as the recognition module. Experimental results showed that the best classifiers are able to obtain average accuracies over 97% on the proposed data set. Vasanthan, V *et al.*, (2019) ^[11] used digital image analysis for identification and discrimination of crop varieties in Sesamum crop and found that 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. Sau, S *et al.* (2018) ^[12] used seed image analysis to discriminate apple germplasm accessions. Digital images of seeds from 42 apple cultivars, acquired by a flatbed scanner, provided a phenotypic dataset with 106 morphometric variables. Stepwise Linear Discriminant Analysis (LDA) was used to examine this dataset, and the results were compared with available genetic data. In agreement with the genetic diversity analysis, the LDA could discriminate between the apples cultivars, identifying two main groups that could be further divided into additional subgroups.

Pereira, C. S *et al.*, (2018) ^[13] proposed a segmentation algorithm based on region growing using color model and threshold techniques for classification of the pixels belonging to vine leaves from vineyard color images captured in real field environment. Concerning boundary-based measures of quality, an average accuracy of 94.8% over a 140 image dataset was achieved. It proves that the proposed method gives suitable results for an ongoing research work for automatic identification and characterization of different endogenous grape varieties of the Portuguese Douro Demarcated Region.

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