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SK Mundotiya
M.Sc. Scholar, Division of Seed Science and Technology, ICAR-IARI, New Delhi, India

Monika A Joshi
Professor, Division of Seed Science and Technology, ICAR-IARI, New Delhi, India

Corresponding Author:
SK Mundotiya
M.Sc. Scholar, Division of Seed Science and Technology, ICAR-IARI, New Delhi, India

Application of digital leaf image analysis to study variation in phenotypically similar extant varieties of rice (*Oryza sativa* L.)

SK Mundotiya and Monika A Joshi

Abstract
Rice (*Oryza sativa* L.) is one of the cultivated cereal plant species, known to have wide diversity and adaptability to growing conditions. An in-depth study of its morpho-physiological characters can improve its yield potential and diversification can be utilized for variety identification under PPV & FR Act 2001. Cultivars are defined by the International Convention for the Protection of New Varieties of Plants (UPOV, 1991). Distinguishing a variety on the basis of classical taxonomic approach is highly difficult because it is time consuming, labour intensive and expensive. Digital image analysis offers an objective and quantitative method for estimation of morphological parameters. Keeping in view the above facts, the present study was initiated with the objective of characterization and to study the variation in phenotypically similar rice varieties using leaf image analysis. The experimental material comprised of twenty-eight extant rice varieties and the sample size consisted of four images for each kind of leaf per side i.e. flag leaf ventral, flag leaf dorsal, penultimate leaf ventral and penultimate leaf dorsal. Thus, total number of images generated were (4 + 4 + 4 + 4) x 28 varieties = 448 images. The generated images were further analyzed for morphological, textural and chromatic features by MATLAB software (version 7.12.0.635, R2011a) and a huge database was created which was further used to generate dendrogram. Cluster analysis was performed based on dendrogram; resulting in seven different clusters of 28 varieties which made differentiation among phenotypically similar rice varieties. Thus, image analysis helped to successfully discriminate the varieties based on the leaf characters of similar rice varieties.

Keywords: Genetic variability, MATLAB software, machine vision, clustering, dendrogram

Introduction
Rice (*Oryza sativa* L.) is one of the cultivated cereal plant species, known to have wide diversity and adaptability to growing conditions. The widespread rice cultivation with extensive contact between people from different regions, its ability to grow under diverse environments and selection pressure, both natural and artificial has led to differentiation of races. Cultivated varieties of rice (*Oryza sativa* L.) are divided in to three sub-group viz. *indica*, *japonica* and *javanica*. All the rice varieties of India and Nepal widely differ from each other in growth duration, photoperiod sensitivity, grain size, shape, and colour and endosperm properties (Ram and Singh 2003) [1] as well as in the level of tolerance/resistance to abiotic and biotic stress (Khus, 1998) [2].

Further study of its morpho-physiological characters can improve its yield potential and diversification can be utilized for variety identification under PPV & FR Act 2001. Cultivars are defined by the International Convention for the Protection of New Varieties of Plants (UPOV, 1991). For characterization, differentiation and protection of varieties specific descriptors are used in each species. Begum and Kumar (2011) [3] characterized thirty-two jute (*Corchorus olitorius* and *C. capsularis*) varieties, including 25 released/notified and seven of common knowledge through distinctness, uniformity and stability (DUS) testing trials for two consecutive years using 17 heritable morphological traits to enable identification of these varieties and for unambiguous ascertainment of distinctness. Raghuvanshi *et al.* (2014) [4] studied *morphogenetic* characterization based on DUS testing, to distinguish seventeen varieties of forage sorghum on the basis of twenty-six essential morphogenetic characteristics, of seedling, plant (vegetative stage and at maturity) and matured seeds, as per the National Guideline for DUS test of Sorghum.

Distinguishing a variety on the basis of classical taxonomic approach is highly difficult because it is time consuming, labour intensive and expensive. Digital image analysis offers an

objective and quantitative method for estimation of morphological parameters. This process uses digital images to measure the size of individual grains and mathematically extract features and shape related information from the images. With the evolution of imaging and computing hardware, several imaging systems were developed for characterization and classification of wheat varieties in US, UK, Canada, and Australia (Varma *et al.* 2013) [5].

Choudhary *et al.* (2007) [6] extracted morphological, color, textural, and wavelet features extracted from colour images of cereal grains for classification Shahin *et al.* (2006) [7] studied size measurements based on image analysis emulating the visual grading system to classify size uniformity in soya bean samples. Visen *et al.* (2000) used algorithms for identification and segmentation of occluding groups of grain kernels in a grain sample image.

Keeping in view the above facts, the present study was initiated with the objective of characterization and to study the variation in phenotypically similar rice varieties using leaf image analysis.

Materials and methods

The field and laboratory experiments were conducted during *kharif* season of 2014-15 in the Division of Seed Science and Technology, ICAR-Indian Agricultural Research Institute, New Delhi. The experimental material comprised of twenty-eight extant rice varieties.

The details with respect to parameters for image capturing and feature extraction are as under:

Image capturing

Nikon D3200 camera was used for capturing the image of object. The height of camera from object was 180 mm (18 cm) and the focus of camera fixed at 0.3 m or 1:3. The distance of light source from the bottom surface of mirror of image set up box was set at 20 cm. The intensity of backlight was 1lux.

Leaf image

Four plants were selected randomly from each variety, uprooted (destructive sampling) and analyzed in divisional laboratory. For leaf imaging, two type of leaves were imaged i.e. flag leaf and penultimate leaf (leaf next to flag leaf) using photography set-up as given in Materials and Methods. In both leaves, both the sides of leaves i.e. ventral (upper) and dorsal (lower) sides were imaged. Hence, the sample size consisted of four images for each kind of leaf per side i.e. flag leaf ventral, flag leaf dorsal, penultimate leaf ventral and penultimate leaf dorsal. Thus, total number of images generated were (4 + 4 + 4 + 4) x 28 varieties = 448 images.

Image processing and extraction of features

MATLAB software (version 7.12.0.635, R2011a) developed by ICAR-CIAE, Bhopal was used for extraction of textural features from the leaf images.

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

Since the objective of the study was characterization and to study the variation in phenotypically similar rice varieties using leaf image analysis, an image library of 448 images was created using flag leaf and penultimate leaf (leaf next to flag leaf) of four plants which were selected randomly from each variety, uprooted (destructive sampling) and analyzed in the divisional laboratory. Leaf images were used to generate huge database related to morphological features (Length, Width, Awn length, Kernel area, Kernel perimeter, Major axis, Minor axis, Eccentricity, Equivalent Diameter, Length-width ratio), textural features (Contrast; Correlation; Energy; Homogeneity; Range; STD; Entropy; Offset 0; Offset 45; Offset 90; Offset 135; SRE; LRE; GLN; LP; RLN; LGRE; HGRE), chromatic features (Redness; Greenness; Blueness; Hue; Saturation; Value; Hue Std;) RHS colour value by using MATLAB software.

The database related to plant leaf images was further processed to generate dendrogram (Fig. 1). Seven different clusters of 28 varieties was formed (Table 1), which showed not only the similarity between varieties but also showed the existing variation with respect to leaf morphological features, textural features and chromatics feature among varieties so as to select most similar group of varieties and also to differentiate the closely related varieties like PB-1 and Improved PB-1.

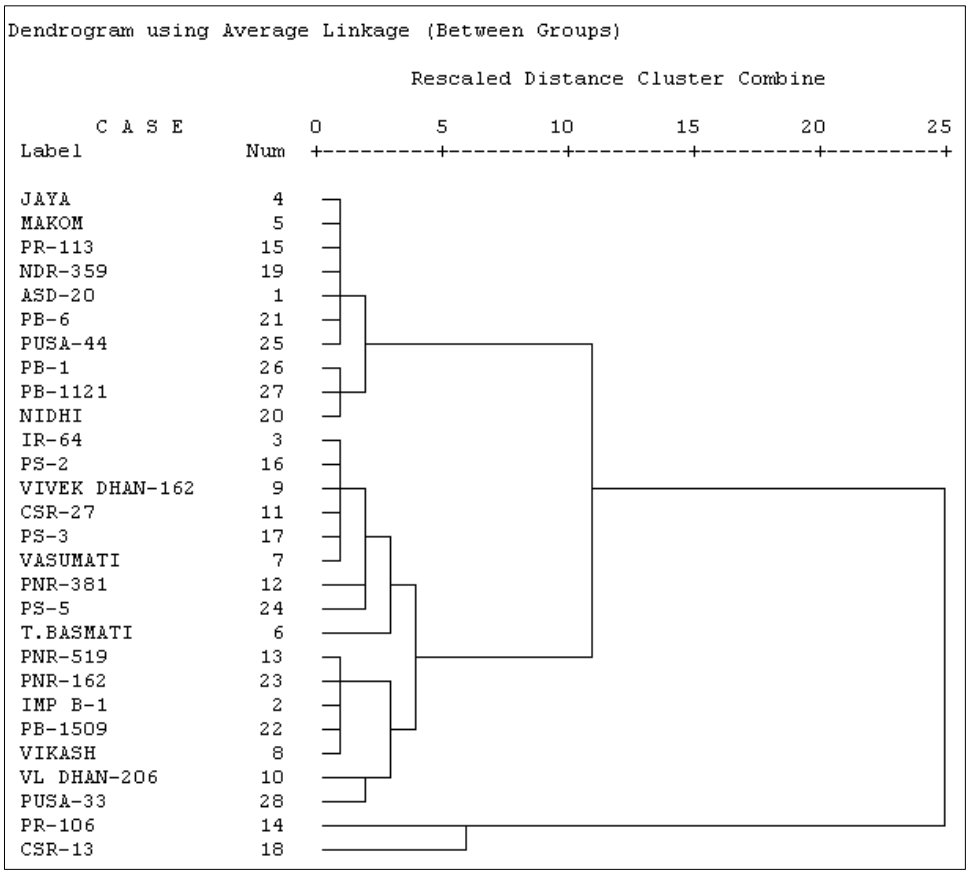

Fig 1: Dendrogram generation based on leaves data.

Table 1: 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

Hence, attention is being laid at international level for the development of suitable lab techniques like image analysis of seed or plant organs, biochemical and molecular markers. Image analysis technique (machine vision system) is one of such systems which offers the prospect that researchers will be able to study 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 and testing of the distinctness of new cultivars for the award of breeders' right and cultivar registration.

Pramanik, S *et al.* (2010) [9] also found that Leaf structures play a very crucial role in determining the characteristics of a plant. The broad and narrow shaped leaves, leaf arrangement, leaf margin characteristics features which differentiate various leaf of a tree. Camelo-Méndez *et al.* (2012) [10] used the result of principal component analysis was an equation with seven variables (area, perimeter, length, width, thickness, sphericity and color), which was useful for distinguishing between nine different cultivars. The morphometric and color parameters for the Mor A-98 and Mor A-92 varieties showed they had 88% similarity. The variability was expressed with a confidence of 95%. Grillo, O *et al.* (2017) [11] experimented with ears of 52 different Sicilian wheat landraces were reaped for three consecutive years. Digital images of the glumes were acquired, processed and analysed, measuring 138 quantitative morpho colorimetic variables. The data were statistically analysed applying a Linear Discriminant Analysis. All the statistical comparisons, distinguished for systematic rank, given perfect identification performances; while an overall percentage of correct identification of 89.7% was reached when all the landraces were compared all together.

Pereira, C. S. *et al.* (2018) [12] 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. Pacifico, L *et al.* (2019) [13] 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) [14] 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 related to genotype and its evolution.

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