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Software-Aided Rice Seed Morphological Characterization for Cultivar Discrimination

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ABSTRACT

The implementation of new methods for reliable and fast identification of seed lot purity and discrimination of one cultivar from another is of major technical and economical importance in the food grain production area of the agriculture industry. As in ocular inspection, the automatic discrimination and grouping of cultivars should be based on knowledge of seed size, shape, color and texture. In this work, we assess the discriminating power of these characteristics for the identification of seeds of 26 rice cultivars. Principal component analysis was complemented by hierarchical clustering with Unweighted Pair Group Method with Arithmetic Mean (UPGMA). In the cluster analysis two major clusters were formed: Aishwarya, ADTRH 1, IR50, CORH2, ADT43, CO43, IR20, ADT37, CO46, ADT38, ASD20 in one major cluster and the remaining cultivars in the other.

Keywords: cultivar identification and purity, image analysis, rice cultivar, seed morphological character, WinDIAS

INTRODUCTION

Cultivar identification is a pre-requisite for the effective provision of Plant Breeders Rights (PBR), which can be achieved by trade secrets, plant variety protection (PVP), or where available through utility patents. All the three forms of protection require some measure of distinctness. Cultivar identification for the attainment of PBR is a taxonomic and genetic approach to determine cultivar distinctness. The chief goals are to promote the release of fresh genetic diversity into agriculture and to create an environment of continued funding for plant breeding research and genetic resource conservation. At the international level, cultivar identification and grain commodity usage become linked, because seeds are the encapsulated intellectual property, the protection of which forms an integral component of the General Agreement on Tariff and Trade (GATT) (Smith *et al.* 1995). Various methods are followed for cultivar identification and seed lot purity determination depending upon the utility of the method, purpose and cost involved. The analysis and classification of cereal seeds are essential activities contributing to the final added value in food grain production. These activities are performed at different stages of the global process, including seed production, cereal grading for industrialization or commercialization purposes, during scientific research for improvement of species, etc. For all these purposes, specialized technicians (Venora *et al.* 2007) are employed. In most cases, these methods are slow, have low reproducibility, and possess a degree of subjectivity hard to quantify, both in their commercial as well as in their technological implications. It is then of major technical and economical importance to implement new methods for reliable and fast identification and classification of seeds. Like ocular identification work, automatic classification should be based on knowledge of seed size, shape and texture. Numerous image-processing algorithms are available for extracting these features from seed images, which make machine vision suitable for such a task (Sako *et al.* 2001; Granito *et al.* 2003). The artificial vision systems are more accurate and efficient in measuring the seed size and textural parameters than inspectors with high experience that work on microscope and purity work board (Churchill *et al.* 1992). In this study, cultivar differences were examined using a seed image analysis system.

MATERIALS AND METHODS

The seeds of 19 rice cultivars (‘CO 43’, ‘ADT 39’, ‘CORH 2’, ‘CR 1009’, ‘ADTRH 1’, ‘ASD 16’, ‘ADT 37’, ‘CO 45’, ‘IR 20’, ‘ASD 20’, ‘ADT 40’, ‘CO 46’, ‘ASD 19’, ‘MDU 5’, ‘TKM 9’, ‘ADT 38’, ‘White Ponny’, ‘IR 50’, ‘ADT 43’) obtained from the Department of Rice, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, 5 rice cultivars (‘Harsha’, ‘Aiswarya’, ‘Kanchana’, ‘Aathira’ and ‘Kairalee’) obtained from Regional Research Station, Pattampi, Kerala and 2 rice cultivars (‘PY1’ and ‘PY 6’) obtained from Krishi Vigyan Khendra, Pondicherry, were used for measuring shape descriptors. The seeds of all 26 varieties were subjected to this experiment, and two replications each of 10 seed were measured. The methodology of instrument usage and parameters studied is described below.

Image analysis

Image analysis was carried out using a Delta –T© (Delta–Instrument Device – Cambridge, UK) image analyzer by running custom written software ‘WinDIAS’.

The experiment was replicated four times, and for every replication 10 seeds were placed in a lighting box in such a way that embryo axes of seed, facing analyzer and longitudinal axes of the seed running parallel to the surface of the camera lens. Seeds were viewed with Video camera (DSP Surveillance colour charged-coupled device (CCD) camera CV-S3200/3300) using transmitted light, so that a binary image of the silhouette of the seed was recorded by the WinDIAS. Images of the seed/embryo were graded by frame grabber to digitize the analogue image and stored in RAM of the computer. The image of the support was removed by software after image grabbing, which thus leaving an image of the objects consist four rows and five column of samples for geometric data measurements.

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Short Communication

Data measurement

Before actual measurement, calibration was done by placing a transparent plastic ruler on the light box illuminated from below. The ruler was aligned diagonally across the camera view and the image was sharpened by adjusting the focus. Again, aperture adjustment was done until optimum color and contrast were achieved. Input length was given in cm.

To measure the descriptors, from the menu tool, area meter was selected. After setting, the image was grabbed using an image grabber and colour thresholds were assigned until the entire area was highlighted. By logging the data, click in the measurement option in the WinDIAS; all descriptor values were extracted each time by clicking individual objects. Values were checked from the review and mean data for the each parameter was summed up for average value in WinDIAS itself. The images were saved in Adobe ImageReady ver. 7.0 as a TIFF file format and their values were saved in Microsoft Excel. The details of measurements made were as follows:

1. Area

2. Perimeter

3. Length

4. Width

5. 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 diameter.

$C = \sqrt{(A/A_p)}$

where, A is the actual area of the object and A_p is the area of a circle with a diameter equal to the circumscribed diameter or length of the object.

6. Elongation
Elongation is the ratio of the length and width of an object. Elongation value recorded for fully matured seeds harvested at physiological maturity stage.

$E = w / l$

7. Shape factor
Shape factor is the ratio of the actual perimeter to that of a circle with the same area.

$S = P / P_C$

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:

$P_C = 2 (\prod * A)^{0.5}$

where, A is the actual area of the object.

8. Average radial
This is an average of all the distances measured from the centroid to each perimeter point.

$R = \sum R_j/n$

where R_j is the j^{th} radius measured from the centroid to the j^{th} perimeter point, and n is the total number of perimeter points.

9. Centroid
The centroid of an object is the most central point or centre of gravity of the object (measured from the top left hand corner of the screen). WinDIAS calculates it using an algorithm based on the following equation.

$\overline{X} = \{1/6 * \sum [(X^2_{i+1} + X_{i+1} * X_i + X_i^2) (Y_{i+1}{}^{-Y_i})]\}/\{ ½ (Y_{i+1} + Y) (X_i - X_{i+1})\}$

$\overline{Y} = \{1/6 * \sum [(Y^2_{i+1} + Y_{i+1} * Y_i + Y_i^2) (X_i - X_{i+1})]\}/\{½ (X_{i+1} + X) (Y_i - Y_{i+1})\}$

10. CMRV
This is the correlation of the average radial and the radial variance.

$CMRV = RV * 100/ R$

11. Radial variance
Radial variance is the square of the standard deviation of all distances measured from the centroid to each perimeter point.

$RV = \sum (R_j - \overline{R})^2/ n\text{-}1$

where R_j is the j^{th} radius measured from the centroid to the j^{th} perimeter point, and n is the total number of perimeter points.

Statistical analysis

Cluster analysis was performed on the similarity matrix based on Jaccard's similarity index by the UPGMA method. All computations were performed with NTSYS-PC ver. 2.1 (Rohlf 1993).

RESULTS AND DISCUSSION

Recent advances in computer image analysis made applicable the approach of automated quantitative analysis aimed to categorize and group cultivars according to slight quantitative differences in seed traits that would be sensorically indiscernible. Quantitative descriptors as continuous variables are supposed to better define cultivar identity and uniqueness on the background of all germplasm in comparison to traditional sensorical descriptors being a categorical variables with substantial decrement of original information. Therefore, using quantitative descriptors more effective economical restructuring and guided reduction of world crop collections may be anticipated (Hammaer *et al.* 2003; Borner 2006).

Quantitative descriptors of seed differences was already effectively exploited in physiological studies (Dell'Aquila 2004), and in cultivar and genotype description, when it helped to more detailed categorizing and grouping of giant ragweed (*Ambrosia trifida* L.) germplasm (Sako *et al.* 2001). Sokefeld *et al.* (1999) demonstrated that it is possible to use computer image analysis to classify seeds. Successfully Variation in seed shape and husk color was investigated in common buckwheat by Tetsuka and Vahino (2005).

In this study, Maximum area (0.18 cm), length (0.94 cm) and perimeter (2.15 cm) was recorded in 'ASD20'. Minimum area (0.11 cm), length (0.65 cm) and perimeter (1.55 cm) was recorded in 'CO 45' (**Table 1**).

Cluster analysis was performed on the similarity matrix based on Jaccards similarity index by the UPGMA method, for performing cluster analysis the recorded mean value of all the cultivars as it is used. In the cluster analysis two

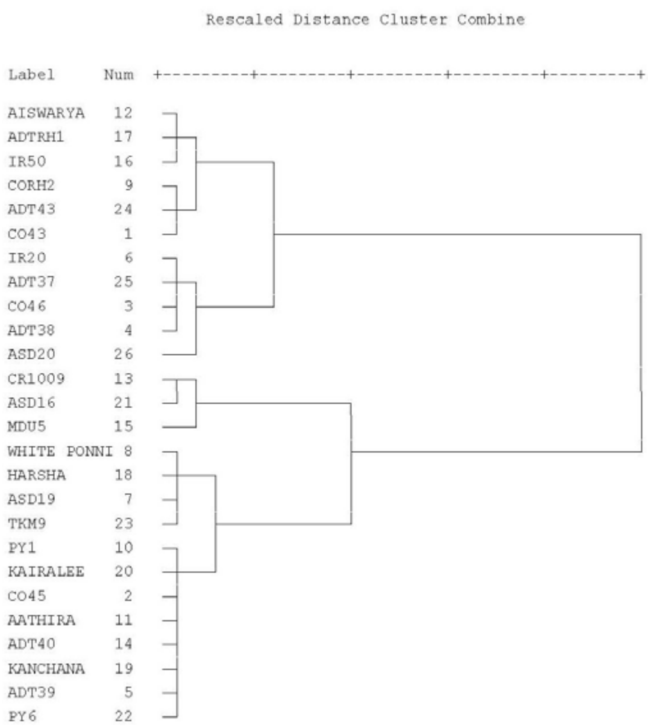

Fig. 1 Dendrogram of 26 rice varieties based on seed image parameters constructed based on Jaccard's similarity index.

Table 1 Mean value (n = 40 per variety) of seed parameters measured through seed image analysis system.

Variety	Area (cm^2)	Centroid X	Centroid Y	Length (cm)	Width (cm)	Average radial	Radial var.	CMRV	Circular	Elongation	S. factor	Perimeter (cm)
CO 43	0.15	3.93	1.95	0.81	0.25	0.24	0.67	274.90	0.54	0.31	1.38	1.90
CO 45	0.11	2.54	3.34	0.65	0.22	0.20	0.40	199.40	0.56	0.34	1.34	1.55
CO 46	0.16	3.18	4.00	0.88	0.24	0.26	0.82	319.30	0.51	0.28	1.44	2.04
ADT 38	0.18	3.35	4.77	0.92	0.26	0.27	0.83	312.50	0.52	0.28	1.40	2.10
ADT 39	0.16	3.07	2.40	0.77	0.27	0.24	0.51	215.40	0.58	0.35	1.30	1.83
IR 20	0.16	2.77	2.33	0.81	0.26	0.26	0.91	324.80	0.56	0.32	1.33	1.90
ASD 19	0.16	3.09	2.05	0.83	0.25	0.33	0.65	239.70	0.54	0.30	1.37	1.94
White Ponni	0.12	3.43	1.92	0.73	0.22	0.22	0.50	229.30	0.54	0.30	1.39	1.70
CORH 2	0.16	2.90	1.41	0.82	0.25	0.24	0.64	262.80	0.54	0.31	1.36	1.91
PY 1	0.13	3.23	2.43	0.70	0.26	0.22	0.41	187.60	0.59	0.37	1.29	1.67
Aathira	0.16	3.43	5.28	0.77	0.27	0.24	0.49	203.54	0.58	0.36	1.31	1.84
Aiswarya	0.15	3.21	1.84	0.86	0.24	0.31	0.77	293.60	0.52	0.28	1.41	1.97
CR 1009	0.14	4.23	1.50	0.66	0.27	0.21	0.30	142.00	0.63	0.41	1.23	1.60
ADT 40	0.16	3.12	2.00	0.76	0.28	0.24	0.50	207.30	0.59	0.36	1.28	1.82
MDU 5	0.16	4.23	1.40	0.75	0.29	0.24	0.40	169.40	0.60	0.38	1.27	1.82
IR 50	0.11	3.23	3.55	0.73	0.20	0.21	0.61	285.90	0.51	0.27	1.46	1.70
ADTRH1 1	0.15	3.21	1.84	0.86	0.24	0.31	0.77	293.60	0.52	0.28	1.41	1.97
Harsha	0.18	2.94	2.51	0.82	0.29	0.25	0.59	231.10	0.58	0.35	1.31	1.94
Kanchana	0.14	3.20	3.97	0.74	0.25	0.23	0.48	209.30	0.58	0.35	1.32	1.77
Kairalee	0.13	4.14	4.88	0.70	0.25	0.22	0.42	191.40	0.59	0.36	1.29	1.66
ASD 16	0.15	4.12	1.94	0.70	0.29	0.23	0.34	148.60	0.63	0.41	1.24	1.73
PY 6	0.11	2.46	2.83	0.68	0.22	0.21	0.44	212.30	0.55	0.32	1.35	1.60
TKM 9	0.16	3.10	3.27	0.73	0.29	0.55	1.16	247.10	0.61	0.40	1.25	1.76
ADT 43	0.13	1.91	2.47	0.79	0.23	0.27	1.16	259.89	0.52	0.29	1.42	1.83
ADT 37	0.16	3.83	1.82	0.90	0.24	0.26	0.86	328.30	0.51	0.27	1.45	2.08
ASD 20	0.18	2.70	2.67	0.94	0.25	0.27	0.94	343.60	0.51	0.27	1.44	2.15

major clusters (**Fig. 1**) were formed. 'Aiswarya', 'ADTRH 1', 'IR 50', 'CORH 2', 'ADT 43', 'CO 43', 'IR 20', 'ADT 37', 'CO 46', 'ADT 38', 'ASD 20', formed one major cluster the remaining varieties formed another cluster. In the final sub-cluster level the following sub-clusters were formed 'Aishwarya', 'ADTRH1' and 'IR50', 'CORH2', 'ADT43' and 'CO43', 'IR20','ADT37','CO46','ADT38' and 'ASD20', 'CR1009', 'ASD16' and 'MDU 5', 'White ponny', 'Harsha', 'ASD19' and 'TKM9', and the following cultivars like 'Kairalee', 'CO45', 'Aathira', 'ADT40', 'Kanchana', 'ADT39' and 'PY6' formed another final level sub-cluster. This experiment shows that in the case of rice, seeds of different rice cultivars were grouped in an objective and quantitative manner. This software-aided imaging technique can be applied to identification of seeds from different species and to study the variability of seed morphology within cultivars.

REFERENCES

Borner A (2006) Preservation of plant genetic resources in the biotechnology era. *Biotechnology Journal* **12**, 1393-1404

Churchill MB, Bilsland DM, Cooper TM (1992) Comparison of machine vision with human measurement of seed dimensions. *Transition of the American Society of Agricultural Engineering* **35 (1)**, 61-64

Dell'Aquila A (2004) Cabbage, lentil, pepper and tomato seed germination monitored by an image analysis system. *Seed Science and Technology* **32**, 225-229

Granito PM, Garralda PA, Verdes PF, Ceccato HA (2003) Boosting classifiers for weed seeds identification. *Journal of Cereal Science and Technology* **3 (1)**, 34-39

Hammaer K, Arrowsmith N, Gladis T (2003) Agrobiodiversity with emphasis on plant genetic resources. *Naturewissenschaften* **90**, 241-250

Rohlf FJ (1993) NTSYS-PC: Numerical taxonomy and multivariate analysis system version 2.1. Exeter Publishing, Setauket NY

SakoY, Regnier EE, Daoust T, Fujimura K, Harrison SK, Mc Donald MB (2001) Computer image analysis and classification of giant ragweed seeds. *Weed science* **49**, 738-745

Smith JSC, Ertl DS, Orman BA (1995) Identification of maize varieties. In: Wringler CW (Ed) *Identification of Food-Grain Varieties*, American Society of Cereal Chemists, USA

Sokefeld M, Gerhards R, Kuhbauch W, Nabout A (1999) Automatic calibration of seeds using digital image analysis. *Agribiol Res Zeitschr Agrarbiol Agrikulturchemie Okologie* **52 (2)**, 183-191

Tetsuka T, Uchino A (2005) Variation in seed shape and husk color in japanese native cultivars of common buckwheat (*Fagopyrum esculentum* Moench). *Plant Production Science* **8 (1)**, 60-64

Venora G, Grillo O, Shahin MA, Symons SJ (2007) Identification of silician Landraces and Canadian cultivars of lentil using an image analysis system. *Food Research International* **40**, 161-166