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ESTABLISHING DISTINCTNESS USING MACHINE VISION FEATURES OF VARIOUS PLANT PARTS IN RICE (*ORYZA SATIVA L*)

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ABSTRACT

Varietal identification is essential for quality seed production. The measurement of geometry of single seed is possible with image analysis. Twenty eight Indian rice varieties were differentiated on the basis of seed morphology and central aerenchymatic structure using machine vision over a period of two consecutive years. Machine vision involved the use of a flat bed scanner for image acquisition and Grain Size and Shape Software for seed image processing. A total of eleven geometric features such as area, perimeter, various length and width parameters, eccentricity, roundness and equivalent diameter were computed on the binary image of seeds. Statistical analysis showed that the precision of machine vision measurements was greater than that of the human measurements. The anatomy and construction of the steler structure for different rice varieties was also studied by binocular microscopy in nodal parts of the rice stem with special emphasis to aerenchyma within the plant stem. Differences in the construction or aerial extend of the aerenchyma between the 28 varieties were observed in central aerenchyma. Thus the study could classify the varieties into separate clusters using shape and size features of both seed and central aerenchyma. Hence, the ability to distinguish between these varieties in present investigation was due to imaging of various plant parts in a particular mode as well as quantitative measurements on geometric and other related features.

***Keywords:* Oryza Sativa L., Scanner; Grain Analysis Software, Binocular Microscope, Shoot-Aerenchyma**

I. INTRODUCTION

Rice is the world's most important food crop and a primary food source for more than one third of world's population. India is endowed with a great diversity of rice germplasm in its vast territorial land area with a variety of short, slender aromatic rice varieties which are popular in different traditional rice growing pockets. Hence, variety identification is a crucial area which traditionally requires skilled human power and is subjective in nature. Also, time required is more and often laborious and costly. Majority of the human activities strongly depend on our ability of visual perception as we use nearly 70% of our brains for visual perception. However, manual operations are considered as inconsistent when operated for long run since human vision is subject to illusions, and quantitatively imprecise. Hence, the manual identification of seeds by specialized technicians is slow, has low reproducibility, and possesses a degree of subjectivity hard to quantify, both in its commercial as well as in its technological implications.

To defeat these practical problems an approach for fast and precise recognition of crop varieties is highly advantageous. Machine vision is a promising technology for rapid identification and automation of grain handling. Several researchers have applied digital image analysis and pattern recognition for objective identification of cereal grains [1-4]. Digital image analysis offers an objective and quantitative method for estimation of morphological parameters. 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 [5-6]. Measurements of various characteristics of wheat grains for cultivar identification, using a video-linked computer system have also been reported [7]. Image analysis is also helpful to evaluate all colours present in given material and is stable as it is referenced against standard colour charts. Besides the use of this method for DUS testing, the derived quantitative colour data can be applied to quantitative genetics for inheritance studies [8].

Production of rice, the world's most important staple food crop, is threatened worldwide by drought. The rice plants require large amounts of mineral nutrients, including nitrogen for their growth, development and grain production. Plants are aerobic organisms and a lack of oxygen presents a severe stress. Low oxygen conditions in plants can be brought about by high metabolic activity, soil waterlogging or flooding [9]. Soil water logging and flooding are environmental conditions that are frequently encountered by plants growing in aquatic or semi-aquatic habitats. However, when plant shoots become flooded, they also experience oxygen shortage. Plants, such as rice (*Oryza sativa L.*), endure frequent flooding by adaptations found not only in roots, but also in stems and leaves. Gas-filled spaces known as aerenchyma are constitutively found in aquatic and semiaquatic plants, and are considered to be an efficient mechanism to ameliorate low oxygen stress. Because most agricultural fields are at least occasionally flooded, the development of crops capable of tolerating flooding by rapidly developing efficient networks of aerenchyma would have important benefits for farmers. Aerenchyma facilitates gas exchange between aerial and submerged plant parts by reducing the diffusion resistance to gas exchange imposed by cells.

Hence, the present study was aimed at resolving differentiation among closely related rice varieties on the basis of grain morphology with the help of machine vision studies and to analyse basic structure of aerenchyma in the rice stem, with an attempt to distinguish the varieties on the basis of these structural differences.

II. MATERIAL AND METHODS

2.1 Plant Material

Twenty eight Indian rice varieties were grown in three replications in the experimental field of Division of Seed Science & Technology, Indian Agricultural Research Institute, New Delhi over a period of two years viz., *kharif* 2013 and 2014. Mature grains were collected and used for recording observations. The grains were cleaned manually to remove non-grain matter and damaged grains. The stem structure was studied to establish distinctness on the basis of central aerenchyma.

2.2 Machine Vision Studies - Seed

For machine vision studies, observations were recorded on ten seeds per variety replicated thrice by three different users; the users were the same who recorded the manual observations. The average readings were thus computed over a period of two years. The total sample size (of 5040 seeds) was the same as for manual observations.

2.3 Imaging Hardware and Software

A flat bed scanner (Canon LiDE 110 version 1.2.00) was used for image acquisition. A Grain Size & Shape Software designed and developed by CIAE, Bhopal was used for the further processing of image.

2.4 Image Generation

Ten rice grains were placed on the scanner avoiding grain to grain contact, thereby circumventing extensive programming needed to separate touching objects and also avoiding associated loss of information in the images. Images of all the grains were grabbed at resolution of 600 dpi. All images were grabbed using the identical settings. The images were stored in *.tif* format for further analysis. The data was replicated thrice for all the varieties using freshly harvested seed from both the years.

2.5 Digital Measurements

For machine vision studies, a digital scanner was used in place of a camera and imaging was done. In the scanner, resolution and distances are fixed, which gives more reproducible results. Inter-laboratory comparisons as well as comparisons with database are better if a scanner is used in place of a camera. ***Eleven parameters*** were defined and measured from the software.

2.6 Central Aerenchyma

Anatomical studies of the aerenchyma system of rice plants was carried out with 25 rice varieties of *Oryza sativa* L. during kharif season of 2014 in the laboratory of Division of Seed Science and Technology, IARI, New Delhi. Structure of aerechyma was studied with binocular microscope. During binocular studies, resolution was so adjusted so as to critically examine the morphological structures of the aerenchyma. The aerenchyma of rice plants was investigated using plants in the reproductive stage. The cross-sections of rice stem were stained with saffranin to observe the detailed structure under microscopy. Images were captured and stored for further study.

III. RESULTS AND DISCUSSION

3.1 Geometry of Seed Using Image Analysis For digital image analysis of rice kernels, a sufficient contrast between the background and the image of the grain is required. For this purpose, the representative sample for each variety was scanned against black background (Fig. 1; i to xxviii). Each grain in the image was detected as per methodology discussed earlier. There was no loss of information during detection and thresholding and the fine details were clearly visible in all the images. The images thus generated were used for further computation of geometric and shape related parameters (Table 1).

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Geometric features such as area, perimeter, bounding box length, bounding box breadth, axial length, axial

Fig. 1 From left to right: i) Basmati 370 ii) CSR 27 iii) Improved PB1 iv) Jaya v) Jyothi vi) Kasturi vii) Kranthi viii) Makom ix) MandyaVijaya x) NDR 359 xi) Pant Dhan 4 xii) Pant Dhan 12 xiii) Pant Dhan 11 xiv) PB1 xv) PB 6 xvi) Phalguna xvii) PR 113 xviii) PS 2 xix) PS 3 xx) PS 5 xxi) PS 4 xxii) Ravi xxiii) Tulasi xxiv) Vasumati xxv) Vijeta xxvi) Vikash xxvii) Vikramarya xxviii) VL Dhan 81

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Table 1: Computation of Geometric Features Based on the Binary Image

S.No.	VARIETY	Geometric features										
		Area (mm^2)	Bounding Box Length, mm	Bounding Box Breadth, mm	Eccentric-ity	Perimeter, mm	Equiv. Dia., mm	Roundness	Axial Length, mm	Axial Width, mm	Median Length, mm	Median Width, mm
1	BASMATI 370	16.6	9.98	2.3	0.972	22.4	4.57	0.212	7.78	2.17	5.34	1.8
2	CSR 27	19.4	9.45	2.74	0.957	21.65	4.97	0.277	6.83	2.61	5.17	2.31
3	IMP. PB 1	17.1	11.07	2.2	0.98	24.63	4.66	0.178	8.36	2	6.93	1.66
4	JAYA	19.6	8.56	3.04	0.93	20.27	5	0.341	5.25	2.97	3.83	2.54
5	JYOTHI	20.8	9.41	2.95	0.94	21.87	5.14	0.299	6.43	2.85	4.58	2.45
6	KATURI	16.6	9.95	2.27	0.974	22.39	4.59	0.215	6.61	2.1	5.59	1.87
7	KRANTHI	19.8	8.29	3.24	0.91	19.72	5.03	0.368	5.39	3.16	3.47	2.64
8	MAKOM	19.6	8.94	2.92	0.94	20.85	4.98	0.312	5.58	2.83	4.28	2.42
9	MANDYA VIJAYA	21.9	9.56	3.05	0.94	22.25	5.29	0.306	5.31	2.95	3.93	2.56
10	NDR 359	21.3	9.6	2.91	0.94	22.11	5.2	0.294	5.94	2.8	5.08	2.48
11	PANT DHAN 11	18.9	8.93	2.81	0.94	20.72	4.91	0.304	5.35	2.7	4.22	2.33
12	PANT DHAN 12	19.7	9.59	2.71	0.95	21.98	5.01	0.273	5.41	2.58	4.04	2.29
13	PANT DHAN 4	21.7	9.49	2.99	0.948	22	5.25	0.308	5.85	2.85	4.44	2.56
14	PHALGUNA	21.3	9.97	2.74	0.962	23	5.21	0.273	5.97	2.63	4.99	2.36
15	PR-113	20.7	9.32	2.89	0.947	21.6	5.14	0.305	5.28	2.8	3.95	2.49
16	PUSA BASMATI 1	17.6	11.00	2.22	0.982	24.49	4.73	0.185	7.13	2	5.63	1.73
17	PUSA BASMATI 6	17.1	11.73	2.04	0.987	25.77	4.67	0.159	10.1	1.9	7.04	1.55
18	PUSA SUGANDH 2	23.9	11.9	2.68	0.975	27.06	5.5	0.215	9.59	2.47	8.34	2.22
19	PUSA SUGANDH 3	23.2	12.23	2.56	0.978	27.23	5.42	0.198	9.34	2.4	7.01	2.15
20	PUSA SUGANDH 4	24.2	12.73	2.5	0.981	28.37	5.53	0.189	8.81	2.36	7.57	2.12
21	PUSA	24.2	12.68	2.59		28.14	5.52		6.51	2.42	4.93	2.13

	SUGANDH 5				0.981			0.193				
22	RAVI	20.9	9.32	2.97	0.945	21.61	5.16	0.308	6.12	2.87	4.45	2.46
23	TULASI	16.9	8.66	2.61	0.95	19.94	4.63	0.29	5.02	2.53	3.85	2.14
24	VASUMATI	20.4	11.31	2.48	0.97	25.27	5.1	0.203	6.96	2.25	5.82	2.03
25	VIJETHA	19.3	9.71	2.64	0.962	22.18	4.97	0.262	5.69	2.51	4.81	2.22
26	VIKASH	19.0	10.17	2.63	0.965	22.93	4.91	0.234	7.7	2.45	4.41	2.09
27	VIKRAMARY A	26.8	10.16	3.53	0.931	23.79	5.84	0.331	7.41	3.47	4.93	2.95
28	VL DHAN 81	20.4	9.76	2.76	0.955	22.32	5.09	0.273	5.58	2.7	4.56	2.37
	MEAN	20.3	10.14	2.79	1	23.07	5.14	0	7.57	2.61	5.11	2.18
	S.D	2.49	1.297	0.499	0	2.34	0.356	0	3.44	0.49	1.31	0.39
	S.E.M	0.47	0.245	0.094	0	0.442	0.067	0	0.65	0.09	0.24	0.07
	VARIANCE	6.23	1.283	0.249	-	5.476	0.127	-	1.77	0.24	1.72	0.15

Varieties Vikramarya (26.802 mm^2) and Kasturi (16.692 $mm^{2)}$ showed the highest and lowest areas, respectively. The varieties differed significantly from each other with respect to all the parameters studied. Bounding box length was highest for Pusa Sugandh 4 (12.73 mm) and minimum for Kranthi (8.29 mm). The varieties were also significantly different from each other with respect to eccentricity, equivalent diameter and roundness. Statistical analysis showed that the precision of machine vision measurements was greater than that of the human measurements and required about one-third of the time. Shouche *et al* [10] calculated Euclidean distance on the basis of differences in geometric and shape related parameters in wheat varieties and concluded that it served as a useful tool for their identification. A similar attempt to measure the shapes of crop and weed seeds by image processing has also been reported earlier by various researchers [11-12]. Based on the results, they concluded that the system was enough to use for classifying and grading the different varieties of rice grains based on their interior and exterior quality.

3.2 Differences In Central Aerenchyma Structure

The oval shaped vascular bundles were observed to be numerous and scattered irregularly in the ground tissue. Peripheral bundles were small and closely arranged. The central bundles were large and widely arranged. In the rice stem, aerenchyma was formed in between the vascular bundles which were located in an outer ring below the epidermis. The conserved number of aerenchyma may be a result of the conserved anatomy of rice with a defined number of vascular bundles in the stem. The number of aerenchyma formed increased from bottom to top in a recurrent pattern that was observed in each internode.

A detailed analysis of the cells that were lysed to form a gas space in the rice stem revealed that these cells possess characteristic features. They were larger, as also observed for cortex cells in rice roots that form aerenchyma [13]. They contained a large vacuole, were lighter in colour and appeared to be devoid of chloroplasts. Unlike the surrounding parenchymal cells, pre- aerenchyma cells contained little or no starch, and had a thin cell wall. The nodes of mature stems were observed to be hollow and additional aerenchyma channels with diameters of 0.1-0.2 mm embedded in parenchymatous cells were found between vascular bundles of the stem walls. The vast aerenchyma system was detectable with in the nodes. Compared to the leaf sheaths enveloping the stem, the extend of the aerenchyma of the stem was much smaller. Differences between the aerenchyma of 25 rice varieties were studied at both vegetative and reproductive stage and the aerenchyma was observed to be well established. The aerenchyma of 25 rice varieties was studied intensively and investigations focused on differences between the varieties. Significant differences were found in the anatomy of the aerenchyma.

An attempt was made to visualise the clustering pattern using shape and size features of central aerenchyma. Six clusters were formed on the basis of similarities in structural features (Table 2). Cluster I consisted of three genotypes, namely, PS 2, PS 3 and PS 5 having elliptical shaped central aerenchyma. Cluster II contained three genotypes-Jyothi, Kranthi and NDR 359 having smaller sized aerenchyma with somewhat elliptical shape as compared to cluster I. Five genotypes- PB 6, PS 4, Imp PB 1, Kasturi and Makom constituted cluster III with circular central aerenchyma of bigger size. Cluster IV included four genotypes namely PB1, Vasumati, Phalguna and Basmati 370 with small and round central aerenchyma. Cluster V included maximum number of genotypes (seven) having varieties Mandaya Vijaya, Vikash, CSR 27, Pant Dhan 4, Vijetha, PR 113 and Vikramarya with clear cut distinguished central aerenchyma. Cluster VI consisted of three genotypes namely VL Dhan 81, Jaya and PD 12 with smaller sized central aerenchyma.

Table 2. Clusters on the Basis of Structural Features of Aerenchyma

CLUSTER	No. OF GENOTYPES	VARIETIES
I	3	PS 2, PS 5, PS 3
II	3	JYOTHI, KRANTHI, NDR 359
III	5	PB 6, PS 4, IMP. PB 1, KASTURI, MAKOM
IV	4	PB 1, VASUMATI, PHALGUNA, BASMATI 370
V	7	MANDYA VIJAYA, CSR 27, PANT DHAN 4, VIJETHA, PR 113, VIKRAMARYA
VI	3	VL DHAN 81, JAYA, PANT DHAN 12

IV. CONCLUSION

The results show the usefulness of machine vision for characterization of various shape and size related parameters of rice. The use of scanner for image grabbing resulted in calculation of various parameters with low coefficient of variation for these geometric features. In addition, several grains can be placed on scanner and a new image is generated where all the grains are arranged in a single row running exactly parallel to each other, enabling quantitative evaluation of individual grains. Statistical analysis showed that the precision of machine vision measurements was greater than that of the human measurements. Hence, the use of over 11 parameters helped to distinguish between 28 rice varieties on basis of grain morphology alone. The study also revealed that by using basic structural differences between aerenchyma, the varieties could be successfully grouped into various clusters. Therefore, the ability to distinguish between these varieties in present investigation was due to imaging of various plant parts in a particular mode as well as quantitative measurements on geometric and other related features.

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