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Characterization of Indian Rice (*Oryza sativa* L.) Varieties based on Seed Morphology and Phenol Colour Test

VIBHA KHANDELWAL, S.K. CHAKRABARTY, MALAVIKA DADLANI AND S.P. SHARMA

Division of Seed Science and Technology, Indian Agricultural Research Institute, New Delhi 110 012
skchakra_sst@yahoo.com

ABSTRACT Characterization of 58 released Indian rice (*Oryza sativa* L.) varieties was done using seed morphological characteristics, *viz*. length, width, colour, shape, presence or absence of awn and phenol colour reaction. Twenty-six varieties were distinguishable individually and the rest were classified into discrete groups. The seed morphological characters based on IRRI and UPOV guidelines including kernel colour and grain length were compared. Phenol colour test, performed on seed from two years and harvest at different locations, confirmed that a specific colour reaction is predominant in each variety, though variation in the intensity of the colour was noticed. A reliable application of positive and negative colour response is suggested. Results suggested that prior to field based testing, these simple laboratory tests could provide for rapid grouping and facilitate verification of a large numbers of rice varieties.

Key words: Rice, varietal characterization, phenol test, seed morphology

The concept of varietal identification has become crucial in view of plant variety protection (PVP), which can be achieved by techniques ranging from simple morphological examination of plant to DNA fingerprinting. Although the choice of technique depends on the purpose of identification, a rational approach would be to employ morphological traits of seed, seedling and mature plant, followed by chemical test, electrophoresis of protein and DNA fingerprinting, sequentially. Characterization of rice varieties is primarily done by morphological characteristics as described in the UPOV test guidelines of rice [1]. This requires extensive replicated field trials in which observations are recorded on at least 50 individual plants at different growing stages. This is both laborious and time consuming. Hence, various approaches were taken to minimize the workload by grouping large number of varieties into several clusters, following simple morphological or laboratory tests.

The test guidelines for stabilizing distinctness, uniformity and stability (DUS) of plant varieties, therefore aims to identify certain stable and prominent characteristics, known to be polymorphic within the given species, as grouping characteristics. Simple chemical tests are known to differentiate plant varieties into discrete classes, *e.g*. phenol test for wheat [2, 3], peroxidase test for soybean, KOH-bleach test for sorghum, fluorescence test for oat [3]. The present study was undertaken to characterize 58 released Indian rice varieties by using seed morphological characteristics and phenol colour reaction, for grouping of varieties, as well as to establish varietal distinctness for DUS testing.

MATERIALS AND METHODS

Authentic seed samples of 58 released Indian rice varieties, in seed multiplication chain, were procured from the respective breeders/breeding centres for examining the seed morphological traits and phenol colour reaction (Table 1). Seeds of 46 varieties were multiplied at Indian Agricultural Research Institute, New Delhi, and at Karnal (Regional Research Station), and multiplied only at New Delhi in *kharif* season,

Table 1. List of rice varieties

Variety	Source
Neela, Heera, Lunisree, Annada Kalinga 3, Tulasi, Vanprava, Ratna	CRRI, Cuttack, Orissa
IR 50, ADT 39, ADT 37, IR 64, CR 1009	TNAU, Coimbatore, Tamil Nadu
Rasi, Jaya, IR 20	UAS, Regional Research Station, Mandya, Karnataka
Govind, Pant Dhan 10, Pant Dhan 11, Pant Dhan 4	G.B. Pant Univ. of Agril. and Tech., Pantnagar, Uttaranchal
Pusa Basmati-1, Pusa 169, Pusa 834, Pusa 205, Pusa 44, Pusa 677, PNR 162, PNR 381	Indian Agricultural Research Institute, New Delhi
Vikas, Swarnadhan, Aditya, Kasturi	Directorate of Rice Research, Hayderabad, A.P.
PR 108, PR 106, PR 111	Rice Research Station, Kapurthala, Punjab
HKR 120, HKR 126, Taraori Basmati, Basmati 370	CCSAU Hissar, Haryana
IR 36, Kranti	IGKVV, Raipur, M.P.
Kushal	AAU, Regional Agri. Research Station, Titabar, Assam
Narendra Dhan 97, Sarjoo 52	NDAUA&T, Faizabad, U.P.
Phalguna, Jyothi	UAS, Regional Agril. Research Station, Mangalore, Karnataka
MTU 2067, MTU 7029, MTU 1001, MTU 5249, MTU 2077	ANGRAU, Hyderabad, A.P.
Red Triveni	Kerala Agricultural University, Trichur, Kerala
Kanak, Intan	Agricultural Research Institute, Mithapur, Bihar
VLD 221	VPKAS, Almora, Uttaranchal
Sona Mahsuri, Sambha Mahsuri	Rice Research Unit, Agricultural College, Bapatla, A.P.

following recommended agronomic practices. Due to poor seed setting and development, the remaining 12 (long duration) varieties released for south India were excluded.

Seed characters

Seed length, width, colour, shape of seed, dehulled grains and presence or absence of awn were recorded in 20 individual grain of each variety. Characterization of seed shape was done as per UPOV guidelines [1, 4]. Grouping of varieties with respect to seed size (length of hulled and dehulled grains) was done, following the IBPGR-IRRI descriptors [5] are presented in Table 2.

Phenol test

Twenty-five grains of each variety were subjected

Table 2. Grain characteristics of rice varieties

IRTF-IRRI scale (mm) for brown rice	FAO scale (mm) for milled rice	USDA worker's scale (mm) for brown rice
Length class (80% sample or more)		
Extra long	≥ 7.50	7.0-7.49	-
Long	6.61-7.50	6.0-6.99	6.6-7.5
Medium	5.51-6.60	5.0-5.99	5.5-6.6
Short	≤ 5.51	≤ 5.0	≤ 5.5

to phenol test, in two replications, after presoaking in distilled water for overnight (17 hr) at 25°C. Presoaked seeds were placed over two layers of filter paper (Whatman No. 1) lined on a (4 inch dia) Petri plate, moistened with 7 ml of 1 per cent phenol solution, incubated at 30°C for 24 hr in dark [6]. Change in seed colour was recorded. One set was maintained as control, in which seeds were kept hydrated in water.

RESULTS AND DISCUSSION

Seed morphology

Based on seed length, 58 rice varieties were classified into three classes, *i.e.* long, medium and short. Maximum number of varieties with hull were 'long' (seed length = 7.6 mm). These were classified under 'long' group, instead of 'extra long' group as per IRRI guidelines. The seed length measurement showed a negligible standard deviation (0-0.4), indicating the stability and reliability of this character. With respect to observable characteristics, three classes were formed under grain width, *i.e.* broad, medium and narrow (hulled and dehulled grains). Maximum number of varieties showed 'medium' grain width. In UPOV guidelines hulled grains were classified into five categories including 'very broad' and 'very narrow'. But observation showed that the above rice varieties could be grouped into three classes only (Table 3).

The seed shape was found to be useful for distinguishing rice genotypes. Both hulled and dehulled grains were classified into four groups, *viz.* very spindle, half spindle, spindle and semi-round. None of the varieties could be grouped in round shape grain as per UPOV. Maximum number of varieties showed 'half spindle' curvature, followed by 'spindle' and 'semi-round' and a very few were observed to have a 'very spindle' shape of the seed, with and without hull.

Colour of grains, with or without hull was also found to be a reliable morphological marker for characterization. Only one variety, Red Triveni, showed red kernel in dehulled seeds. In UPOV test guidelines of rice, grain colour is not included, but one Indian variety was distinguished based on grain colour (*e.g.* Red Triveni). Presence or absence of awn was

Table 3. Number of rice varieties classified into different groups based on seed size

A. Length	Long	Medium	Short
Seed with hull	(≥ 7.6 mm) 38	(6.1-7.5 mm) 15	(5.0-6.0 mm) 5
Dehulled seed	(≥ 7.0 mm) 22	(5.0-6.9 mm) 34	(< 5.0 mm) 2
B. Width	Broad	Medium	Narrow
Seed with hull	(≥ 3.0 mm) 7	(2.5-3.0 mm) 31	(< 2.5 mm) 20
Dehulled seed	(≥ 2.5 mm) 10	(2.0-2.5 mm) 44	(< 2.0 mm) 4

recorded as one of the distinct marker (Table 4). However, it was found to be useful only when seed are not processed.

Table 4. Classification of rice varieties based on seed colour, shape and awn

Seed shape	Very spindle	Half spindle	Spindle	Semi round
Hulled Seed	3	30	18	7
Dehulled grain	2	38	7	11
Seed colour	Light brown		Variegated brown/red	
Seed with hull	49		9	
Dehulled seed	51		7	
Awn	Present		Absent	
	9		49	

Phenol test

Several researchers have demonstrated the efficacy of phenol colour reaction to differentiate between rice varieties [7-11]. In the present study, results of this test were determined predominantly on the basis of colour reaction as light brown, dark brown and no colour (Table 5). 41 varieties were classified under light brown, 12 under dark brown and only five with no colour group, though a lot of intra-varietal variability was seen in the first two classes. Further studies were carried out only with 46 varieties, which showed a relatively consistent colour reaction. Of these 46 varieties, for which seeds were multiplied in two seasons at two locations, 36 were light brown and five each were in dark brown and in no colour group. Data recorded on seed samples from two locations and one location, showed a range of variability in colour intensities in each class. Cent per cent uniformity was observed only in no colour class, whereas 65.5-93.5 per cent variability was noted in light brown and 68.0-76.5 per cent in dark brown classes.

Majority of varieties (36) fell into light brown class with a mean of 82.34 per cent and a standard deviation of 4.41. The mean and standard deviation were 76.1 and 2.94 for dark brown and 100, 0 for no colour classes, respectively. Thus maximum variability of colour intensity was observed within the light brown rice group. In a similar study on 27 rice varieties, it was observed that intra-varietal colour variations occurred in 25 varieties, both in standard as well as modified phenol test and hence is not useful for characterization of rice varieties [6].

The results showed that there is an inherent variability within varieties with respect to colour intensity obtained in phenol test, which was

Table 5. Grouping of 58 rice varieties* based on phenol colour reaction

Light brown	Dark brown	No colour
Neela, Jaya, Heera, Phalguna, Kalinga III, Pusa Basmati 1, Vanprava, PR 106, ADT 37, PR 111, Rasi, Kasturi, Govind, PD 10, Annada, Pusa 169, Vikas, Pusa 205, Kranti, Pusa 834, IR 36, Pusa 677, PNR 162, PD 11, Ratna, Kanak, IR 64, Red Triveni, VLD 221, PR 108, Aditya, IR 50, ND 97, PNR 381, HKR 120, HKR 126, PD 4**, Pusa 44**, MTU 1001**, Swarnadhan**, Lunisree**	ADT 39, Tulasi, MTU 7029, MTU 2077**, Sona Mahsuri, Kushal, IR 20**, Sarjoo 52**, Intan**, Jyothi**, CR 1009**, Samba Mahsuri**	MTU 2067, MTU 5249, MTU 5293, Taroari Basmati, Basmati 370

*Procured from breeders/breeding institute; **Varieties showed inconsistent phenol colour reaction

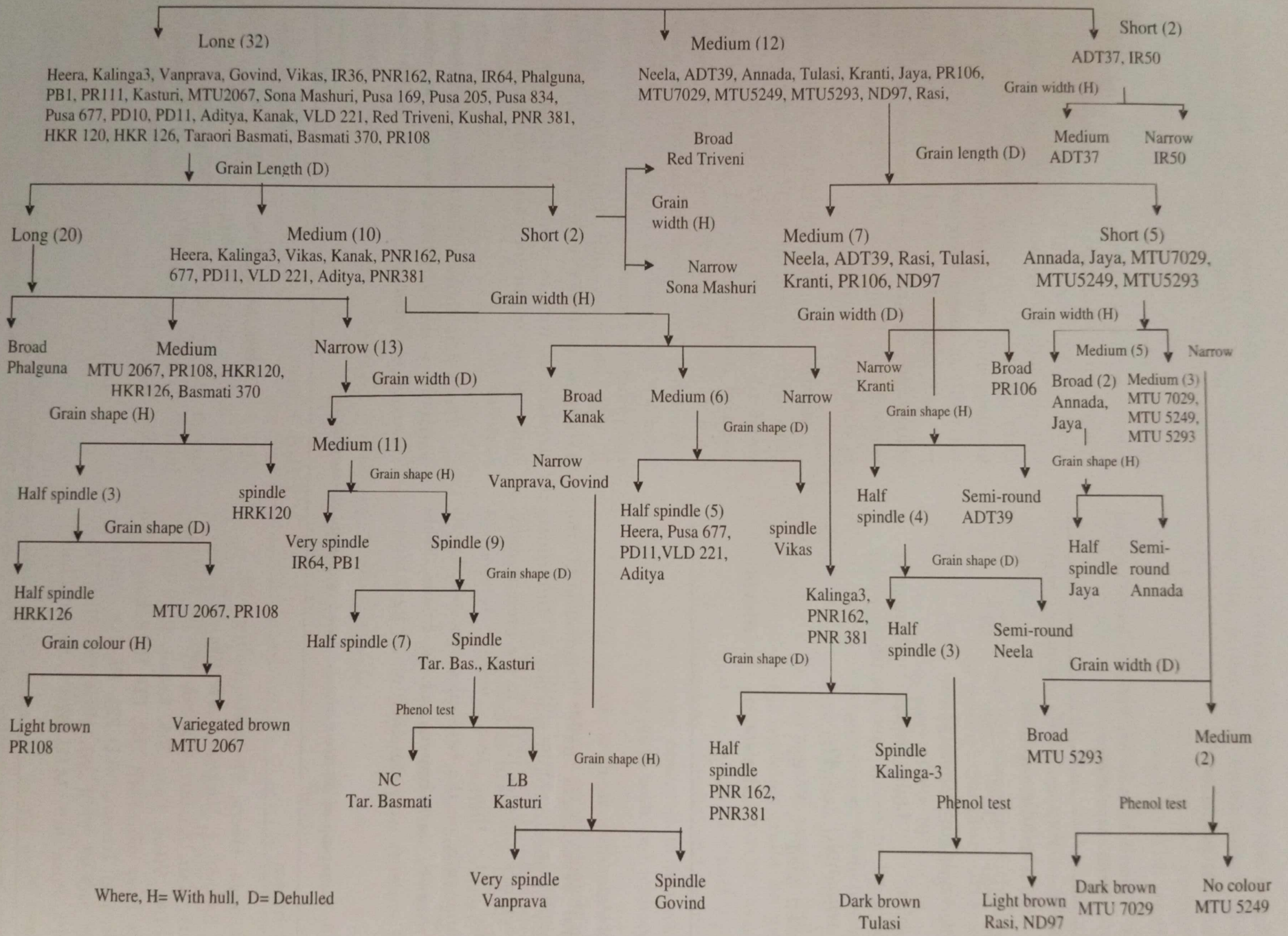

Fig.1. Identification of forty-six rice varieties based on seed morphological characters and phenol colour reaction

highest in seed stock obtained from the breeders and it declined in the successive generations. The non-uniformity of colour reaction observed in rice, as against wheat could be assigned to factors such as the growing environment. When dehulled seeds are subjected to phenol test little or no response was recorded. Thus, flavanoids known to be present in the husk [12] may be playing an important role in development of colour intensity. Though the oxidation of phenolics is known to be catalyzed by tyrosinase group of enzyme [13], its intensity may depend on non-enzymatic factors as well.

Both inter-and intra-panicle variability with respect to colour intensity was tested in individual panicles of all the varieties. Though there was no intra-panicle variability, inter-panicle variability ranged from 0 per cent (Kalinga 3, IR 20, Aditya) to as high as 50 per cent (Vikas, HKR 120, Sona Mahsuri). This suggest that phenol colour reaction in rice is possibly influenced by non-genetic factors such as germination status, age (date of harvest), location of production and seed health, which need further investigation.

The revised UPOV Guidelines (2002) for DUS testing in rice include phenol reaction and classifies varieties into dark brown, brown, light brown and no colour groups. However, in National DUS Test guidelines (PPV&FRA), only two states of expression (0 & 1) are suggested for phenol colour reaction. Present results support that only a positive or negative phenol colour test response may be used for grouping or distinguishing rice varieties, irrespective of intensity of colour developed; the colour intensity, is a highly variable criterion.

In addition to DUS test, phenol test could be useful for seed quality control. Taroari Basmati and Pusa Basmati-1, the two popular commercial varieties can easily be differentiated by phenol colour reaction; Taroari Basmati exhibited a negative colour response, whereas Pusa Basmati-1 showed a light brown colour.

An identification key was prepared (Fig. 1), classifying 46 rice varieties on the basis of characters studied. Twenty-one varieties were distinguishable simply on the basis of seed morphological characters, *viz.* length, width, shape and colour (both hulled and dehulled). Awn character was excluded, as its reliability will be in question, once the seeds are processed. Inclusion of phenol colour test further resolved the identities of five more varieties and thus, 26 varieties in all could be identified. Thus, by using seed morphological characters and phenol test, we can make grouping and verification of a large number of varieties for varietal distinctness, prior to field trials. For confirmation of the grouping, the tests should be followed by a detailed examination of plant characters in field trial.

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