Seed Research, Vol. 35(2): 215-219, 2007

Variation in Phenol Colour in Relation to Age of Rice Seeds

S.K. CHAKRABARTY, K.C. NANDI AND MALAVIKA DADLANI

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

ABSTRACT Identification of plant varieties, based on morphological, physiological or biochemical characteristics, is of great significance in variety development, protection, seed production and certification programmes. Keeping this in view, 49 released varieties of rice were tested and characterized on the basis of phenol colour reaction. Intra-varietal differences and the effect of physiological status of seed were determined in fresh and one year old seeds of all varieties. The study revealed that the above rice varieties could be grouped into five discrete colour groups by phenol test. However, 19 varieties out of 49 showed intra-varietal variation in the colour intensities, where as it was consistent in the remaining 30 varieties. The varieties exhibiting no colour change were most consistent with respect to phenol reaction with 100 per cent reproducibility. The intensity of the phenol colour reaction deepened with seed age in 14 out of 19 lots. Thus, it is concluded that phenol test may be performed on fresh seeds and the test should be used only to classify varieties as colour positive (light brown, brown, dark brown and black) and colour negative (no colour).

Key words: phenol colour, rice varieties, seed age

Characterization and verification of crop varieties are essential components of variety development, seed production as well as plant variety protection system. Intensive crop breeding programmes, the world over, have resulted in the development of a large number of varieties in all major crops. In India, the highest number of varieties has been released in rice, among the crop species. In addition to these, there are a large number of local varieties and land races available which are adapted to diverse agro-climatic conditions of India. Characterization of large number of varieties based on morphological characteristics of the plant (or plant part) is often not sufficient to discriminate between varieties. Hence, several rapid and simple chemical tests have been developed for grouping of plant varieties.

Phenol colour reaction is one such test, based on tyrosinase enzyme catalysed oxidation of phenol into dark coloured melanin, which has been widely used for determining varietal purity in wheat varieties [1, 2, 3, 4]. The phenol test has been found to be useful for rice and pearl millet also and there are reports suggesting that phenol colour test can also be used for varietal identification in rice [5, 6, 7]. However, not much information is available regarding the reliability of this test in rice and the effect of physiological status of the seed in colour development. Keeping this is view, 49 released varieties of rice were tested for phenol colour reaction in the present study. Fresh and one year old seed lots of the 49 varieties were subjected to phenol colour reaction and intra-varietal differences with respect to colour development were determined.

MATERIALS AND METHODS

The seed materials for the present study consisted of 49 varieties of rice (Table 1). These varieties were collected from respective breeders of various Breeder Seed Production (BSP) units of ICAR Institutes and State Agricultural Universities. These seed materials were grown in the *kharif* season of 2001 at IARI research farm, New Delhi for further multiplication. The harvested crop along with the original seed lot were used for the study. Two replications of twenty-five seeds of each variety

Received December 2006 Revised June 2007 Accepted July 2007

were presoaked in distilled water overnight (~ 17 hours) at 25°C. Two layers of filter papers (Whatman No. 1) lined on the Petri plates (4 inches diameter) were moistened with 7 ml of 0.1 per cent (v/v) phenol solution. Premoistened seeds were placed equidistantly over this in Petri plates and fitted with lids. One set (25 seeds) of samples was maintained as control, in which seeds were kept hydrated in water. The samples were kept in dark (incubator) at 30°C for 24 hours for colour development, after which the seeds were examined for phenol colour reaction on their husks (lemma and palea) and grouped on the basis of colour intensity as, no colour development (-), light brown (+), brown (++), dark brown (+++) and black (++++) [8].

RESULTS AND DISCUSSION

The variation in phenol colour intensity groups in the two seed lots of 49 varieties are presented in Table 1. The phenol reaction resulted in light brown, brown, dark brown and black colouration in the seeds of 46 varieties. There was no colouration in three varieties, i.e., ADT 39, Basmati 370 and Taraori Basmati. In the 46 varieties, showing colour reaction, there was variation in the intensities among and within the two seed lots. The varieties, therefore, were grouped on the basis of the predominant colour development.

The predominant phenol colour remained almost same in 30 out of the 49 varieties tested in the two seed lots (Table 2). However, percentage of seeds occurring in a particular colour group including the predominant one varied between the two seed lots. The varieties (ADT 39, Basmati 370 and Taraori Basmati) showing no colour reaction were absolutely uniform in their expression of both seed lots. Red Triveni showed the highest percentage (88-96%) of seeds with brown (++) colour group in both seed lots.

Nineteen varieties showed variation in number of seeds showing predominant colour group in the two seed lots studied (Table 3). The colour groups ranged from light brown (+) to dark brown (+++). In 14 out of these 19 varieties the older seed lots gave darker reaction compared to the corresponding fresh seed lots.

The phenol test, which is an established method for grouping of wheat varieties as well as for testing varietal purity, did not show intra-varietal variation for colour classes [2, 3, 9]. Intra-varietal variation for two seed colour classes was reported in seven cultivars out of 14 rice cultivars [6]. In the present study also out of 49 cultivars, in 46 cultivars there were two or more seed colour classes, suggesting that the phenol colour intensity groups are of limited use in characterization of varietal purity in rice.

The reasons for intra-varietal variation for phenol colour in rice may be that there was no selection made for this trait in the breeding material and during the variety development the character expression is incomplete; and there could be physiological reasons for such variation. But the study clearly showed that since most of Indian rice cultivars are showing intra-varietal variation for phenol colour intensity, this method is not suitable for varietal characterization in rice. Possibly this might be the reason why this method could not find a place in ISTA rules for verification of cultivars in rice, although investigation on phenol colour test started as early as 1940 [10]. While in case of wheat it has been found useful and was incorporated in ISTA rules as early as 1966 [11].

It was also noteworthy in the present study that the extreme colour groups represented in lower frequency as compared to the moderate colour groups. This possibly hints to more than two genes controlling the character with incomplete penetrance.

From the results it is suggested that phenol colour test for variety characterization and if used in DUS testing, it should be done on fresh seeds only either using the submitted seed samples or with fresh seeds harvested from the crop grown for DUS testing to validate the results and for establishment of uniformity and stability. The varieties should only be grouped as (i) phenol colour positive(+), encompassing all varieties exhibiting light brown to dark brown/black colour intensities and (ii) phenol colour negative(–) those showing no change in colour.

The present investigation also showed that

Table 1. Phenol colour reaction on seeds (in percentage) of 49 rice cultivars in the two seed lots

Cultivar	2001 (old)					2002 (fresh)				
	Black (++++)	Dark brown (+++)	Brown (++)	Light brown (+)	No colour (–)	Black (++++)	Dark brown (+++)	Brown (++)	Lignt brown (+)	No colour (–)
VL Dhan 221	0	0	34	66	0	0	0	26	74	0
Heera	0	4	30	66	0	0	6	48	46	0
Kalinga 3	0	4	30	62	4	4	14	40	42	0
Vanaprava	2	18	36	44	0	0	10	38	52	0
Pant Dhan 11	0	32	52	16	0	2	30	42	26	0
Narendra 97	2	26	34	36	2	6	34	26	30	4
Govind	2	14	32	52	0	4	22	38	36	0
Annada	8	14	42	36	0	2	24	44	28	2
ADT 37	14	24	40	22	0	12	36	26	26	0
RASI	0	10	14	76	0	2	8	24	66	0
Pusa 169	0	14	48	38	0	4	10	40	46	0
Pusa 677	0	4	48	48	0	6	4	46	44	0
IR 36	0	24	48	28	0	0	2	44	54	0
Redtriveni	0	6	88	4	2	0	4	96	0	0
IR 50	2	2	52	44	0	2	2	54	34	8
Ratna	2	4	36	52	0	0	6	32	62	0
Aditya	6	26	36	28	4	0	22	38	40	0
PNR 162	2	10	48	36	4	0	10	46	44	0
Pusa 834	4	6	50	40	0	0	10	58	28	4
Vikas	26	32	22	20	0	0	10	44	46	0
Jyothi	16	36	28	20	0	0	20	76	4	0
PNR 381	0	10	66	24	0	4	2	44	50	0
PR 111	0	20	60	20	0	0	2	54	44	0
Neela	6	16	40	38	0	0	4	38	54	4
Pant Dhan 10	6	24	56	14	0	14	32	34	20	0
PR 108	4	32	48	16	0	0	52	34	14	0
Kasturi	0	8	42	50	0	0	4	48	48	0
Jaya	6	36	30	28	0	0	6	64	30	0
Pusa 205	0	32	36	32	0	0	24	42	34	0
HKR 126	4	46	28	16	0	8	42	20	30	0
Pusa Basmati 1	0	26	54	20	0	0	2	60	38	0
PR 106	0	14	64	22	0	0	6	44	48	2
Basmati 370	0	0	0	0	100	0	0	0	0	100
Pant Dhan 4	2	34	48	16	0	0	44	36	20	0
Pusa 44	2	52	30	16	0	2	14	60	24	0
HKR 120	4	18	62	16	0	2	10	66	26	0
Kranti	4	48	26	22	0	8	28	54	10	0
Phalguna	8	24	40	28	0	0	26	56	18	0
Kanak	4	34	48	14	0	0	8	62	30	0
Taraori Basmati	0	0	0	0	100	0	0	0	0	100
Sona Mahsuri	0	36	30	34	0	12	36	36	16	0
ADT-39	0	0	0	0	100	0	0	0	0	100
Sarjoo 52	26	46	18	10	0	0	18	60	22	0
IR 20	16	56	10	18	0	18	38	40	4	0
Swarna Dhan	10	40	22	28	0	0	34	48	18	0
Intan	72	28	0	0	0	56	44	0	0	0
Kushal	28	52	12	8	0	26	60	10	4	0
Tulsi	84	16	0	0	0	54	28	18	0	0
Lunisree	60	24	16	0	0	44	34	22	0	0

Table 2. The cultivars showing seeds with similar predominant phenol colour reaction in two seed lots

Colour group	Cultivars	2001(old)	2002(fresh)	Mean	Remarks
Black (BL) (++++)	Intan	72	56	64	28-44% DB
	Tulsi	84	54	69	16-28% DB to B
	Lunisree	60	44	52	16-34% DB to B
Dark brown(DB) (+++)	HKR126	46	42	44	4-30% BL to LB
	Sona Mahsuri	36	36	36	12-36% BL to LB
	Kushal	52	60	56	4-28% BL to LB
Brown (B) (++)	PantDhan 11	52	42	47	2-32% BL to LB
	Ananda	42	44	43	2-36% BL to NC
	Pusa 677	48	46	47	4-48% DB to LB
	Red Triveni	88	96	92	2-6% DB to NC
	IR 50	52	54	53	2-44% BL to NC
	PNR 162	48	46	47	2-44% BL to NC
	Pusa 834	50	58	54	4-40% BL to NC
	PR 111	60	54	57	2-44% DB to LB
	Pant Dhan 10	56	34	45	6-32% BL to LB
	Pusa 205	36	42	39	24-34% DB to LB
	HKR 120	62	66	64	2-26% BL to LB
	Phalguna	40	56	48	8-28% BL to LB
	Kanak	48	62	55	4-34% BL to DB
	Pusa Basmati-1	54	60	57	2-38% DB to LB
Light brown (LB) (+)	VL Dhan 221	66	74	70	26-34% B
	Heera	66	48	57	4-46% DB to B
	Kalinga 3	62	42	52	4-40% DB to B
	Vanparava	44	52	48	2-38% BL to DB
	Rasi	76	66	71	2-24% BL to B
	Ratna	52	62	57	2-36% BL to B
	Kasturi	50	48	49	4-48% DB to B
No colour (NC) (-)	Basmati 370	100	100	100	-
	Taraori Basmati	100	100	100	-
	ADT-39	100	100	100	-

three cultivars namely, Basmati 370, Taraori Basmati and ADT 39 showing no colour change could be characterized and identified by phenol colour test along with other characteristics.

REFERENCES

1. CSALA, M.V. (1972). The methodology and mechanisms of the phenol reaction in cereals. *Proc. Intl. Seed Test. Assoc.*, **37**: 915-921.
2. STEEN, K.M., O.L. KARSKY & J.D. MAGUIRE, (1986). A method for quantitative colour of phenol reaction on wheat seed. *J. Seed Technol.*, **10**: 62-67.
3. SINGHAL, N.C. & S. PRAKASH (1989). Use of phenol colour reaction on seeds and outer glumes for identification of bread-wheat cultivars. *Plant Varieties and Seeds*, **1**: 153-157.
4. ISTA. (2004). International rules for seed testing. *Seed Sci. & Technol.*, **21** (Supplement): 1-288.
5. ABROL, Y.P. & D.C. UPRETY (1972). Phenol colour reaction vis-a-vis discolouration of paddy. *Bull. Grain Tech.*, **10**: 288-290.
6. SIVASUBRAMANIAN, S. & V. RAMAKRISHNAN (1974). Use of phenol colour reaction for identification of rice varieties. *Exp. Agric.*, **10**: 299-303.

Table 3. The cultivars showing differences in percentage seeds showing predominant phenol colour reaction groups in two seed lots

Cultivars	2001 (old)		2002 (fresh)	
	Colour group	No. seeds(%)	Colour group	No. seeds(%)
Narendra Dhan 97	Light brown	36	Dark brown	34
Govind	Light brown	52	Brown	38
ADT 37	Brown	40	Dark brown	36
Pusa 169	Brown	48	Light brown	46
IR 36	Brown	48	Light brown	54
Aditya	Brown	36	Light brown	40
Vikas	Dark brown	32	Light brown	46
Jyoti	Dark brown	36	Brown	76
PNR 381	Brown	66	Light brown	50
Neela	Brown	40	Light brown	54
PR 108	Brown	50	Dark brown	52
Jaya	Dark brown	36	Brown	64
PR 106	Brown	64	Light brown	48
Pant Dhan 4	Brown	48	Dark brown	44
Pusa 44	Dark brown	52	Brown	60
Kranti	Dark brown	48	Brown	54
Sarjoo 52	Dark brown	46	Brown	60
IR 20	Dark brown	56	Brown	40
Swarnadhan	Dark brown	40	Brown	48

7. WANG, J. & Z. SHEN (1992). Study on the classification of American rice varieties. *Crop Genetics Resources*, **2**: 4-6.

8. GUPTA, P.K. & R.L. AGRAWAL (1988). Determination of varietal purity of paddy varieties by laboratory evaluations. *Oryza*, **25**: 310-314.

9. WALLS, F.W. (1965). A standardized phenol method for testing wheat for varietial purity. *Handbook on Seed Testing*. Contribution No. 28, Assoc. of Official Seed Analysts.

10. KONDO, M. & Y. KASAHARA (1940). On the discrimination of rice varieties by means of colouring reaction of hulled kernels to phenol-fuschine. *Proc. Crop. Sci. Soc.*, Japan, **12**: 122-128.

11. ISTA. (1966). International rules for seed testing. *Seed Sci. & Technol.*, **24** (Supplement): 1-335.