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Variation in phenol colour reaction in grains of rice (*Oryza sativa* L.) varieties

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Abstract

Phenol colour reaction on grains and glumes is used for characterization of rice varieties for distinctiveness, uniformity and stability (DUS) testing in plant variety protection. According to DUS test guidelines, rice varieties are classified into two groups based on presence and absence of phenol colour reaction on the grains. However, rice grain changes to black or light brown colour in different frequencies in seed lots of varieties. An investigation was conducted to differentiate 20 rice varieties based on variations in phenol colour reaction. Studies were also conducted to show intra-panicle and inter-plant and panicle variation in paddy seeds based on the variation in phenol colour response during different seed growth and maturity stages. The results showed a negligible existence of inter- and intra-plant/panicle variation in grain in rice varieties due to phenol colour reaction. The phenol colour reaction starts at the post fertilization stage of the spikelet and is maintained in the matured grains. Instead of two groups the phenol colour test in the existing DUS test guidelines a higher number (five) of groups like black, dark brown, brown, light brown and no colour is recommended as a grouping characteristic in DUS testing of rice varieties for plant variety protection.

Keywords: Phenol colour test, stage of induction, variation, DUS testing, rice variety

Introduction

The release of a large number of rice varieties and hybrids has increased the task of breeders and seed technologists to ensure the quality of seed. Varietal descriptions given by the breeders most often relate to plant morphological characters that are not sufficient to identify varieties or seed lot adequately. Identification of varieties at the seed or seedling level using phenol test, modified phenol test; ferrous sulphate test, potassium hydroxide test, sodium hydroxide test and GA_3 chemical tests is rapid, reliable and reproducible in cereals and vegetables. Traditionally, characterization and identification of a variety is done based on morphological characters, recorded during full growth stages. The morphological characters are the universally undisputed markers for varietal characterization and identification. A small variability observed between two varieties results from the narrow genetic base of the germplasm/parental lines used by the breeders, the environmental effect on the expression of the characters and the time required for obtaining results. Therefore, additional descriptors that are not influenced by the environment and with significant discriminating power and reproducibility are needed like rapid chemical test (Kumar et al. 2013).

Besides a large number of landraces, about 1741 improved varieties have been notified for cultivation since 1966 till April, 2020 in India. It has been assessed that 1 per cent adulteration in the seed reduces down the potential yield of a variety about by 100 kg ha^{-1} (Sripunitha and Sivasubramaniam 2014). In a country like India, where contract farming is adept at many places for seed production with the active involvement of the private sector (Mishra et al. 2003), monitoring genetic purity at each stage of seed production becomes necessary. In this view, the study on varietal characterization becomes imperative. Realizing this, glume phenol reaction (phenol test) of grain has been included as one of the characteristics for Distinctiveness, Uniformity and Stability (DUS) testing in rice under DUS test guidelines by both UPOV

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and PPVFRA (ISTA 1990, 1993). Moreover, DUS characterization of cultivars is also required for their protection according to the Protection of Plant Varieties and Farmer's Right Act, 2001 (PPV & FR Act 2001). Varieties can be identified using morphological and chemical tests. Among them, chemical tests are quick, easy to carry out, reproducible and can be undertaken throughout the year under controlled conditions. As chemical reaction is based on the chemical nature of proteins which are products of genes, chemical testing, therefore, is specific to the genetic nature of the crop. Some of the sensitive tests employed in the laboratory are phenol test, modified phenol test; ferrous sulphate test, potassium hydroxide test, sodium hydroxide test and GA_3 test (Sripunitha and Sivasubramaniam 2014).

The phenol colour test, which is an index of polyphenol oxidase activity, is a simple method for grouping the rice varieties (Oka 1958; Abrol and Uprety 1972; Vanangamudi et al. 1988; Sivasubramanian and Ramakrishnan 1974). During phenol color test, phenol gets oxidized into dark colour melanin pigment catalyzed primarily by the enzyme tyrosinase present on the seed coat and is under simple genetic control in wheat (Joshi and Banerjee 1970; Bhowal et al. 1969; Nair and Tomar 2001). In this test, seed or glume colour reaction serves as a basis for grouping varieties. Phenol colour response has been extensively used as a rapid laboratory test for governing varietal purity in wheat. There is ample literature (Casla 1972) on phenol colour responses in wheat. The approaches have been systematic and give congruous results. It was set up in ISTA, 1966. Wheat varieties of Indian origin and accessions of different ploidy levels were tested by Singhal and Prakash (1988), Singhal et al. (1991) and Nair and Tomar (2001) for phenol reaction on grains and glumes and categorized them into four groups as black, dark brown, brown and light brown colour; none of the genotype was non reactive (negative reaction) to phenol. A large number of notified Indian durum wheat varieties have been characterized for phenol reaction by different researchers (Kundu et al. 2006; Rajender et al. 2009). Niranjana et al. (2018) recorded phenol reactions among genotypes in wild species, bread wheat cultivars, durum wheat and synthetic hexaploids (SH) to breed a wheat variety, that do not react to phenol and does not darken the dough. The inheritance study showed that development of phenol colour in grains was governed by three dominant alleles.

The phenol reaction described whether rice hulls and grains darken after exposure to a 1-2% aqueous phenol solution; *japonica* varieties showed no change in colour (a negative response), whereas *indica* varieties and wild *Oryza* species changed to dark brown or black coloration as a result of polyphenol oxidase (PPO) activity (a positive response) (Yu et al. 2008). Results from different colour groups in response to phenol reaction showed an inherent variability within varieties with respect to colour intensity. Such variation was attributed to be due to predisposition of different environmental conditions during growth (Khandelwal et al. 2010). Intra-varietal phenol colour reaction to grains/seeds has also been reported earlier (Chakrabarty et al. 2007; Khandelwal et al. 2010).

Phenol reaction, controlled by a single gene, is localized in seed coat, therefore, it was considered as an important primary descriptor for grouping and identification of germplasm lines of rice. The gene underlying the phenol reaction has been cloned and characterized or identified in *O. sativa*. The phenol-negative response characterizing or identifying *japonica* varieties was shown to be the result of three different loss-of-function mutations (sudden heritable change) at the *Phr1* locus, which encodes the PPO enzyme (a protein in the tyrosinase family). The majority of *japonica* lines sequenced to date contain an 18-bp deletion in exon 3 that results in a non-functional (*phr1*) allele; a minority have either a 29-bp deletion in exon 3 or a 1-bp insertion in exon 1, which also result in non-functional alleles and negative phenol reactions (Yu et al. 2008).

In view of the fact that phenol colour reaction has been recommended as a characteristic in the DUS testing system for grouping of varieties and owing to the possible inconsistency in test results in some groups of varieties, two states of expression *i.e*, present and absent was considered. Therefore, it is imperative to understand the source of variability in grains/seeds of varieties of different phenol colour groups in relation to stage of induction of phenol colour, maturity of grains and inter- and intra-plant differences. Based on the above background a research work was envisaged to understand the occurrence of phenol colour reaction in terms of its induction since anthesis and to assess the source of variation among varieties for phenol colour reaction.

Materials and methods

Materials

The phenol colour reaction was studied in 60 varieties of rice released in India. The seeds were produced

following plant to progeny method of multiplication during *kharif* season, 2019 in the field at ICAR- Indian Agricultural Research Institute, New Delhi. Based on the phenol colour reaction test, 4 varieties each (from a set of 60 varieties) in 5 colour groups were selected to carry out other experiments (Table 1).

Table 1. A list of varieties with their phenol colour reaction

Black	Dark Brown	Brown	Light Brown	No colour
Krishnaveni	MTU1010	Satabdi	Falguna	Vandana
CSR13	PNR519	Pant Dhan-4	Mahamaya	Satyabhama
CRD300	Kranti	Jaya	IR-64	VL Dhan 206
PNR381	Swarna	PR-113	ADT-37	Triguna

Phenol colour test method

Two replicated samples of 50 grains of each variety were soaked in distilled water for 16 hours at the temperature of 25°C±1°C in the incubator. Later, the grains were flushed with tap water and excess water was removed using a blotter paper. Two layers of filter paper were placed in a plastic Petri plate of diameter 11 cm and moistened with 5 ml of 2% phenol solution. 50 seeds were randomly placed on the two layers of filter paper. The Petri plate was then closed and kept in dark in an incubator at 25^{0}C. After 4 hours of incubation, the number of seeds that took colour due to phenol in each variety was recorded. This method has been standardised by us (Kumar 2020).

Experiment 1

Freshly harvested grains in *kharif,* 2019 (grown from single plant seeds produced in *kh*arif, 2018 and following plant to progeny method) and after drying for 5 days in oven at 35^{0}C, the varieties were tested for phenol colour response. Another lot of seeds of the same varieties grown in *kharif,* 2018, kept at ambient room temperature (referred to as 2018) were also used for the studies.

Experiment 2

In *kharif,* 2019, the panicles of the main tiller of 15-20 plants of each of the selected 4 varieties in each colour group indicated above were labelled at the time of flower opening. Glumes/grains of 3 plants in the main tiller was collected at different stages of its growth and maturity as follows: stage 1: before anthesis/ opening of spikelet (glumes); stage 2: 10 days after anthesis (milk stage); stage 3: 20 days after anthesis (dough stage) and stage 4: 30 days after anthesis (harvest maturity stage). It is to be noted that glume at stage 1 consisted of empty, unfertilized spikelet with lemma and palea. The dried glumes or grains were used for study of phenol colour reaction.

Experiment 3

Seeds of the selected varieties (Table 1) were collected from the main tiller, side tiller and late tiller in 3 plants of each variety at harvest maturity stage. Similarly, seeds of the main tiller of 3 plants in each variety were separated into 3 equal parts i.e., basal, middle and top portion. This was done in panicles collected at stages 2, 3 and 4 as indicated in Experiment 2.

Results and discussion

Phenol colour response of rice varieties

Freshly harvested grains of 60 rice varieties grown in *kharif* 2019 (grown from *kharif* 2018 single plant to progeny) were tested for phenol colour response. The fresh seed lots of 13 varieties in black colour group showed a mean of about 83% (range: 63-100) and with 9, 1 and 3 varieties showing >90, 80-90 and <80% seeds, respectively, with black colour. Similarly, in dark brown, brown and light brown colour groups, the number of varieties with more than 90% were 0/9, 6/ 11 and 4/16, respectively; between 80-90% it was 7/ 9,5/11 and 11/16, respectively while in less than 80% group it was 2/9, 0/11 and 1/16 varieties, respectively (Table 2). In no colour group, the varieties showed a mean of 93 % grains showing negative phenol colour response with a range of 64-100%. Nine and 2 varieties in no colour group (11) showed >90% and <80 % seeds with no colour change, respectively (Table 2). The reason for the variation in colour reaction among the grains in some of the varieties could be due to genetic impurity and /or physical admixture of the varieties. A very high frequency of seeds responded to the phenol colour reaction belonged to black and no colour groups.

Table 2. Variation in phenol colour reaction (%) in the different phenol colour reaction group varieties in fresh grains

Colour group	No. of varieties	Range	Mean	Name and number of varieties with coloured seeds		
				>90%	80-90%	<80%
Black	13	63-100	83.03	Krishnaveni, CSR 13, CRD 300, PNR 381, ADT-20, Ranikajor, Kalakheri, Budi luchai and Surajone (9)	IR-24 (1)	Sonam, Mandya vijaya and PR-106 (3)
Dark Brown	9	54-90	79.9	-	MTU-1010, PNR-519, Kranti, Swarna, Aruna, Dudheswar, HMT(PKV) (7)	Bhadra and Suraksha (2)
Brown	11	54-94	78.20	Satabdi, Pant Dhan-4, PR-113, Jaya, PB-1 and Baspatri(6)	PB-6, IR-8, Gontra -2, Rasi and Kudrat (5)	-
Light Brown	16	75-97	87.06	Falguna, Mahamaya, IR 64 and ADT 37(4)	Khandagiri, NDR-359, Ramaya, JD-13, Improved PB-1, Shyamla, PS-4, Pusa 44, Ravi, CRD-204 and Vikas (11)	Annada (1)
No colour	11	64-100	93.22	Vandana, Satyabhama, VL Dhan-206, Triguna, Lochit, Makom, Vivek Dhan, Govindbhog, Jaisree, Lunisree and Vijetha (11)	-	-
Total	60			30	24	6

In the brown colour groups the predominant colour group was evident with some grains showing other colour groups. It could be due to differential maturity and accumulation of chemicals responsible for the colour reaction response. It can be concluded that phenol colour reaction in the varieties is a highly genetically controlled trait. Oka et al. (1958) reported that this trait was controlled by a single gene (*Phr1*).

Phenol colour reaction of grain at different growth stages and maturity

Seeds in panicles harvested at properly developed four different stages were tested for phenol colour response. In the first stage (before anthesis of spikelets), no colour response was observed except in a few black colour varieties like Ranikajor, and Surazone originally having black glumes colour. Clancy et al. (1982) conducted an experiment to determine the stage of seed development at which phenol test can be carried out for cultivar identification. Several varieties were chosen for their study and harvests were made at 3 to 4 days interval from 7-10 days post-anthesis to full seed maturity. They concluded that the phenol test could be used for identification purposes once the seed reached the hard dough stage and the chlorophyll content had declined. When the chlorophyll content of the chaff was high, very little stain in green wheat seed was produced by phenol. The authors thought that the chlorophyll in the seed was perhaps masking the brown colouration produced by the phenol reaction. In the second stage *i.e.,* about a week after anthesis or just after fertilization showed development of colour with a high degree of intensity. In third stage (dough stage) and fourth stage (harvest maturity), intensity of colour development was low but evenly distributed over the paddy seeds. Though spikelet is formed during the panicle development, it did not respond to phenol prior to fertilization. Therefore, some grain formation and its growth factors must be involved in its colour reaction to the spikelet/husk/glume.

McCallum and Walker (1990) did analysis of soluble phenolics in wheat and suggested that the maximum content of soluble phenolics per seed occurred 35-40 days after ear emergence and that their concentration in the immature grain was higher. Content of soluble phenolics was maximal just prior to the appearance of mature grain colour and when grain volume was also at a maximum. The fact that development of mature grain colour was associated with a marked decline in phenolics supports the idea that seed coat pigments may be the products of their oxidation.

Percentage of seeds in phenol colour response was about 95% in general in each colour group. The varieties showed a very high value in changed colour of seeds across the stages (Table 3) which has been be quantified by total phenol content and their intermediates which results in colour development (Kumar 2020). The results indicated that the varieties studied had high purity in respect of the phenol colour response to the seeds at varied growth and maturity conditions. However, a significant difference in the percentage of seeds with phenol colour response was recorded among the varieties and at different stages of growth (Table 3). Variation in colour pattern between seed lots of the same wheat variety has also been reported. Therefore, seeds may be classified as durum, white or red wheat prior to conducting the phenol test and varieties in each wheat class should be recorded as staining either light or dark. This would greatly reduce the chance of misinterpreting variations among seed lots of the same cultivar.

Several researchers have tested the potency of phenol reaction to discriminate among rice varieties. Studies were conducted and different categories of phenol colour reaction formed. Majority of varieties have shown light brown colour, having higher mean

Table 3. Phenol colour reaction in grains at different stages of growth and maturity in varieties of different colour groups

Colour group	Variety	Coloured seeds (%)				
		Stage 1	Stage 2	Stage 3	Stage 4	Mean*
Black	Krishna veni	0(0)	97.6(81.4)	95.6(77.9)	96.3(79.1)	96.5(79.5)
	CSR 13	0(0)	96.3(78.9)	94.6(76.6)	95.6(78.0)	95.5(77.8)
	CRD 300	0(0)	95.3(77.5)	96.3(79.2)	96.3(79.1)	96.0(78.6)
	PNR 381	0(0)	96.6(79.6)	92.0(74.3)	94.6(76.6)	94.4(76.8)
Dark Brown	MTU1010	0(0)	93.0(75.0)	94.0(75.9)	94.6(76.6)	93.8(75.8)
	PNR 519	0(0)	98.0(81.8)	97.3(80.7)	96.6(79.6)	97.3(80.7)
	Kranti	0(0)	96.3(79.1)	94.6(76.6)	95.6(77.9)	95.5(77.9)
	Swarna	0(0)	95.0(77.0)	96.6(79.5)	94.6(76.7)	95.4(77.7)
Brown	Satabdi	0(0)	96.3(79.1)	97.3(80.7)	94.6(76.7)	96.1(78.8)
	Pant Dhan-4	0(0)	94.6(76.6)	96.3(78.9)	94.3(76.4)	95.1(77.3)
	Jaya	0(0)	93.3(75.2)	93.6(75.4)	93.0(74.8)	93.3(75.1)
	PR113	0(0)	93.3(75.0)	94.6(76.6)	98.0(81.8)	95.3(77.8)
Light Brown	Falguna	0(0)	97.6(81.4)	97.0(80.6)	96.6(79.5)	97.2(80.5)
	Mahamaya	0(0)	96.6(79.5)	95.0(77.0)	93.6(75.5)	95.1(77.3)
	IR-64	0(0)	96.0(78.4)	94.6(76.6)	97.0(79.9)	95.8(78.3)
	ADT 37	0(0)	95.0(77.0)	94.0(75.8)	95.6(77.9)	94.8(76.9)
No colour	Vandana	0(0)	96.0(78.4)	91.6(73.2)	90.0(71.8)	92.5(74.5)
	Satyabhama	0(0)	91.6(73.5)	90.6(72.3)	93.3(75.0)	91.8(73.6)
	VL Dhan 206	0(0)	91.(73.2)	98.0(81.8)	98.6(81.1)	95.8(78.9)
	Triguna	0(0)	95.6(77.9)	96.0(78.4)	97.0(79.9)	96.2(78.8)
	Mean	0(0)	95.3(77.8)	95.0(77.1)	95.3(77.7)	

CD (p=0.05); Var:1.94; stage:0.662; var x stage=3.36

Figures in parenthesis indicate transformed values; analysis did include data obtained at stage 1, *: mean is of stage 2-4

and standard deviation than dark brown and no colour and thus, it was clear that light and dark brown colour shows variation in intensity of colour developed**.**

Inter-panicle variation for phenol colour reaction at maturity

Mature seeds of the middle portion of panicles *i.e.* main tiller (panicle 1), mid-tiller (panicle 2) and late tiller (panicle 3) of a plant were analysed for phenol colour reaction in all the selected 20 varieties. The results indicated that the varieties differed significantly in colour reaction but those do not show any significant variation in phenol colour reaction in the grains among the three different panicles of varying emergence and maturity of a plant (Table 4). This showed non-existence of intra-plant or inter-panicle variation in a plant of a variety (Figs. 1 and 2).

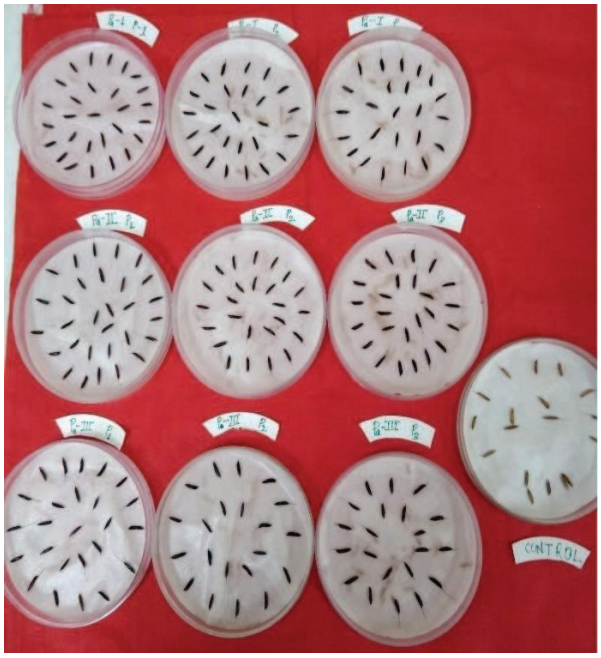

Fig. 1. Inter-panicle phenol colour reaction

Further, the four varieties in each colour group also did not show any difference in the per *cent* grain colour reaction among the three panicles (Table 4). Also within a colour group the varieties did not show any inter-panicle difference in the phenol content (Kumar 2020).

Intra-panicle variation for phenol colour reaction at different stages of growth and maturity

In order to understand the phenol colour reaction induction and its further development with respect to grain growth and maturity the panicles were labelled and harvested at various stages since starting of panicle emergence (pre-anthesis) till harvest maturity of panicles. The results indicated that grain colour reaction was not obtained in the panicles at pre-anthesis stage. The varieties showed positive phenol colour reaction in the stage 2, 3 and 4. The varieties showed higher intensity of coloured grain in the stage 2 *i.e.*,10 days after anthesis (Table 5).

The results indicated that there is some effect of the grains positioned at different parts of the panicle. The grains at the top of a panicle showed significantly higher percentage (96.4) of colour change across the colour group varieties. Similarly, the seeds at the middle portion of a panicle at the stage 3 (dough stage)

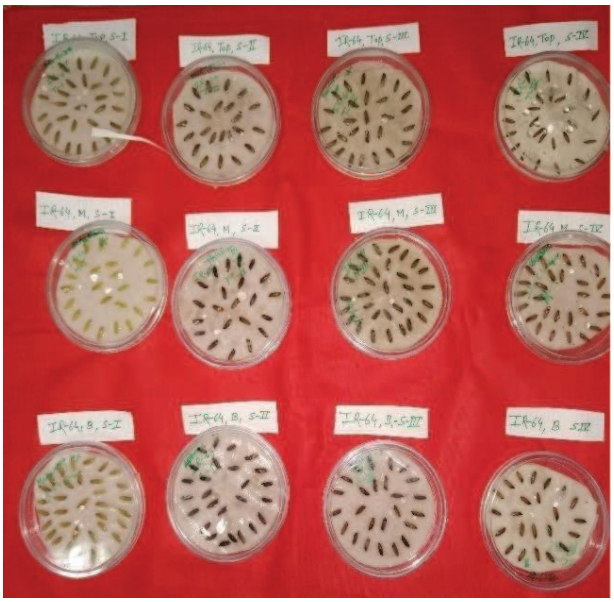

Fig. 2. Intra-panicle phenol colour reaction

had a higher percentage (96%) of grain colour change across the colour groups (Table 6). At maturity stage (stage 4) the seeds at the basal portion showed significantly higher percentage (96%) colour change compared to the top and middle portions (Table 7). The higher number of grains in particular stage and position in a panicle could be due higher accumulation of poly phenol oxidase and its intermediate chemicals in the biosynthesis pathway to melanin (Kumar 2020). Intra-panicle variation was due to seeds at the basal part of a panicle which were possibly immature and chaffy spikelets. Intra and inter-plant phenol colour reactions across the colour groups are attributed to

Table 4. Phenol colour response in the grains borne in three panicles of a plant in varieties of different colour groups

Colour group	Variety	Seeds (%) in			
		Panicle 1	Panicle 2	Panicle 3	Mean
Black	Krishna veni	94.3(76.2)	95.6(78.0)	95.3(77.5)	95.1(77.2)
	CSR 13	94.3(76.5)	94.3 (76.3)	94.6 (76.8)	94.4(76.5)
	CRD 300	94.3(76.4)	95.3 (77.6)	94.0(75.9)	95.3(76.6)
	PNR 381	94.6 (77.0)	94.6(76.6)	96.0(78.4)	95.5(77.3)
Dark brown	MTU1010	94.6 (76.6)	95.6(78.0)	95.0 (77.9)	95.3 (77.5)
	PNR 519	95.6 (78.0)	98.3 (82.6)	98.0 (82.0)	97.3 (80.8)
	Kranti	98.6 (83.4)	96.3 (79.2)	95.6 (78.0)	96.8 (80.2)
	SWARNA	96.3(79.2)	96.3 (79.1)	95.6(78.4)	96.1 (78.9)
Brown	Satabdi	95.0(77.0)	96.6(79.4)	95.3 (77.8)	95.6 (78.1)
	Pant Dhan-4	95.6 (78.2)	94.6(76.6)	94.6(76.7)	95.0 (77.2)
	Jaya	95.0(77.0)	96.3(79.1)	90.3(75.1)	94.8 (77.1)
	PR113	96.0(78.4)	95.6(78.2)	95.0(77.0)	95.5(77.9)
Light Brown	Falguna	98.0 (82.0)	98.6(83.4)	99.0(84.2)	98.5(83.2)
	Mahamaya	96.6(80.0)	97.0(80.4)	95.0(77.1)	96.2(79.2)
	IR-64	94.6(76.8)	97.0(80.0)	96.3(78.9)	96.0(78.6)
	ADT 37	94.3 (76.2)	94.0 (75.8)	94.6 (77.0)	94.3(76.3)
No Colour	Vandana	95.3(78.1)	95.0 (77.0)	95.0 (77.4)	95.1 (77.5)
	Satyabhama	94.0 (75.9)	95.3 (77.5)	94.0 (75.9)	94.4 (76.4)
	VL Dhan 206	96.6 (79.6)	96.3 (79.4)	98.0(82.0)	97.0 (80.3)
	Triguna	98.3(82.8)	99.0(84.2)	96.6(79.8)	98.0(82.2)
	Mean	95.6 (78.3)	96.1 (78.9)	95.6 (78.2)	
CD(p=0.05): Var: 2.40; Panicle: NS; V×Pa: NS					

Figures in parenthesis indicate transformed values

Table 5. Phenol colour response in the grains (%) located in different positions in a panicle at stage 2 (post-anthesis) of a plant in varieties of different colour groups

Variety	Top	Middle	Basal	Mean
Krishna veni	95.5(77.7)	96(78.4)	95(77.0)	95.5(77.7)
CSR 13	96.5(79.2)	95.5(77.7)	94(75.8)	95.3(77.5)
CRD 300	95(77.1)	95.5(77.7)	95(77.3)	95.1(77.3)
PNR 381	97.5(80.9)	95(77.1)	95(77.1)	95.8(78.3)
MTU1010	97(80.1)	95.5(77.7)	97(80.1)	96.5(79.3)
PNR 519	94(75.7)	97(80.1)	96(78.4)	95.6(78.1)
Kranti	96(78.5)	96(78.8)	94.5(76.7)	95.5(77.7)
Swarna	95.5(77.7)	97(80.1)	95(77.1)	95.8(78.3)
Satabdi	97(80.1)	94(75.9)	94(75.7)	95(77.3)
Pant Dhan-4	97(80.1)	94(75.7)	96(78.4)	95.6(78.1)
Jaya	95.5(77.7)	97(80.1)	95(77.1)	95.8(78.3)
PR113	97(80.1)	94(75.7)	96(78.4)	95.6(78.1)
Falguna	97(80.1)	95.5(77.7)	97(80.1)	96.5(79.3)
Mahamaya	97.5(80.9)	95(77.1)	95(77.1)	95.8(78.3)
IR-64	97(80.1)	95.5(77.7)	97(80.1)	96.5(79.3)
ADT 37	95.5(77.7)	96(78.4)	95(77.0)	95.5(77.7)
Vandana	96.5(79.2)	95.5(77.7)	94(75.8)	95.3(77.5)
Satyabhama	97(80.1)	94(75.9)	94(75.7)	95(77.3)
VL Dhan 206	97(80.1)	94(75.7)	96(78.4)	95.6(78.1)
Triguna	97(80.1)	95.5(77.7)	97(80.1)	96.5(79.3)
Mean	96.4(79.1)	95.3(77.6)	95.3(77.7)	
CD(p=0.005): var: NS; position: 0.59; var x position: NS				

Figures in parenthesis indicate transformed values

Table 6. Phenol colour response in the grains (%) located in different positions in panicle at stage 3 (dough stage) of a plant in varieties of different colour groups

Variety	Top	Middle	Basal	Mean
Krishnaveni	94(75.8)	96(78.4)	95.5(77.7)	95.1(77.3)
CSR 13	94.5 (76.4)	96(78.5)	96(78.4)	95.5(77.7)
CRD 300	93.5(75.2)	95.5(77.7)	95(77.1)	94.6(76.6)
PNR 381	95.5 (77.7)	97(80.1)	95.5(77.8)	96(78.5)
MTU1010	95.5 (77.7)	96.5(79.2)	96.5(79.4)	96.1(78.7)
PNR 519	97(79.9)	95.5(77.8)	95(77.1)	95.8(78.3)
Kranti	96(78.4)	95.5(77.8)	96.5(79.4)	96(78.5)
Swarna	92(74.9)	95(77.0)	97(80.1)	94.6(77.3)
Satabdi	94(75.8)	96.5(79.4)	96(78.4)	95.5(77.9)
Pant Dhan- 4	93(74.9)	96(78.8)	95(77.1)	94.6(76.9)
Jaya	92(73.3)	95(77.0)	97(80.1)	94.6(77.3)
PR113	93(74.9)	96(78.8)	95(77.1)	94.6(76.9)
Falguna	95.5(77.7)	96.5(79.2)	96.5(79.4)	96.1(78.7)
Mahamaya	95.5 (77.7)	97(80.1)	95.5(77.8)	96(78.5)
IR-64	95.5 (77.7)	96.5(79.2)	96.5(79.4)	96.1(78.7)
ADT 37	94 (75.8)	96 (78.4)	95.5 (77.7)	95.1 (77.3)
Vandana	94.5 (76.4)	96 (78.5)	96 (78.4)	95.5 (77.7)
Satyabhama	94 (75.8)	96.5 (79.4)	96 (78.4)	95.5 (77.9)
VL Dhan 206	93 (74.9)	96 (78.8)	95 (77.1)	94.6 (76.9)
Triguna	95.5 (77.7)	96.5 (79.2)	96.5 (79.4)	96.1 (78.7)
Mean B	94.3 (76.5)	96.0 (78.6)	95.8 (78.4)	
CD(p=0.005): var; NS; position: 1.02; var x position: NS				

Figures in parenthesis indicated transformed values

Table 7. Phenol colour response in the grains (%) located in different positions in panicle at stage 4 (harvest maturity) of a plant in varieties of different colour groups

Variety	Top	Middle	Basal	Mean
Krishna veni	94.6 (76.6)	94.6(76.6)	94.0(75.8)	94.4(76.3)
CSR 13	95.6(77.9)	96.0(78.4)	97.0(79.9)	96.2(78.8)
CRD 300	95.3(77.5)	93.0(75.6)	94.3(76.2)	94.2(76.4)
PNR 381	93.3(75.2)	93.0(75.6)	93.3(75.2)	93.2(75.3)
MTU1010	96.0(78.4)	95.6(77.9)	96.0(78.4)	95.8(78.3)
PNR 519	94.6(76.7)	94.6(76.6)	94.3(76.2)	94.5(76.5)
Kranti	93.3(75.2)	96.0(78.4)	95.0(77.1)	94.7(76.9)
Swarna	95.3(77.6)	94.6(76.6)	96.3(79.1)	95.4(77.8)
Satabdi	96.0(78.4)	96.0(78.5)	95.6(78.0)	95.8(78.3)
Pant Dhan-4	92.0(74.0)	96.0(78.5)	96.0(78.6)	94.6(77.1)
Jaya	92.0(74.0)	96.0(78.6)	96.0(78.4)	94.6(76.6)
PR113	96.3(79.1)	96.0(78.4)	95.0(77.1)	95.7(78.2)
Falguna	95.3(77.6)	96.0(78.6)	96.0(78.6)	95.7(78.3)
Mahamaya	96.0(78.4)	95.6(77.9)	94.6(76.7)	95.4(77.7)
IR-64	94.6(76.7)	94.6(76.6)	95.6(77.9)	95.0(77.1)
ADT 37	96.6(79.5)	94.6(77.0)	96.0(78.6)	95.7(78.4)
Vandana	94.0(75.9)	94.6(76.6)	96.0(78.6)	94.8(77.0)
Satyabhama	94.6(76.6)	95.6(77.9)	94.6(76.6)	95.0(77.1)
VL Dhan 206	95.6(77.9)	95.6(77.9)	94.6(76.6)	95.3(77.5)
Triguna	94.0(75.9)	94.6(76.6)	95.6(77.9)	94.7(76.8)
Mean	94.7(77.0)	95.1(77.5)	95.3(77.5)	
CD(p=0.005): var: NS; position: 1.1; var x position: NS				

Figures in parenthesis indicate transformed values

the polyphenol oxidase activity estimated in different stages of grain maturity and grains in different panicles in a plant and its position in a panicle (Kumar 2020).

Presently based on the phenol colour reaction in grains of rice varieties are classified into two groups (present and absent). In the absence of inter- and intra-plant and panicle variation in seeds due to phenol colour response, the fresh seeds harvested at harvest maturity may be used for phenol colour test in grains as a characteristic with 5 states of expression (i.e., black, dark brown, brown, light brown and no colour) in DUS testing of rice varieties. This will widen the scope of plant variety characterization for its identification and further protection.

Authors' contribution

Conceptualization of research (SKC); Designing of the experiments (SKC, SK); Contribution of experimental materials (SKC); Execution of lab experiments (SK, YS); Analysis of data and interpretation (SK, SKC); Preparation of the manuscript (SK,SKC).

Declaration

The authors declare no conflict of interest.

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