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Chemical, Biochemical and Seed Composition Assays for Characterisation of Paddy Genotypes

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Abstract: *Raichur is coming under northern dry zone of Karnataka and is known as Rice bowl of Karnataka. Identification of paddy genotypes is of prime importance to ensure quality seed, which is required for achieving global food demand. Ten important paddy genotypes well suitable which are adjusted to this region were identified by knowing their response to different chemicals (Phenol, Modified phenol with Copper sulphate, $FeSO_4$, NaOH, KOH and Peroxidise) tests. All the genotypes were identified by using key based chemical tests. Phenol, Modified phenol with Copper sulphate $FeSO_4$ and KOH tests were able to distinguish all the genotypes. In peroxidase test, the genotypes did not show any peroxidase activity which was considered as absent. All the genotypes showed yellow colour to NaOH test. Chemical tests could be used as a powerful tool to identify every genotype in a short period of time.*

Keywords: *Paddy, Chemical test, varietal identification key, phenol.*

INTRODUCTION

Paddy is the most important staple food crop of more than 60 per cent of the world population. Paddy provides about 22 per cent of the world supplies of calories and 17 per cent of the proteins. Maximum area under paddy is in Asia. Total estimated area under paddy production in the world is 156 million hectares with a production of 650 million tones and the average yield is 3689 kg per hectare (Anon., 2012). To sustain its high production and productivity, a number of high yielding varieties and hybrids have been developed and notified in the recent past, out of which many varieties and hybrids are now in seed production chain. The release of large number of rice hybrids has increased the task as well as the responsibilities of seed technologists in order to ensure the quality of seed. Seed technologists must be well equipped to identify different varieties and hybrids, both at field and at seed level. Varietal descriptions given by the breeders most often relate to field characters and not sufficient to identify genotypes or seed lot adequately. The alternative way to speed up the testing procedures is to use chemical tests in place of morphological markers. These chemical tests are very quick, easy to do, reproducible and can be undertaken throughout the year under controlled conditions.

While electrophoresis is the most widely used biochemical test for identification and characterization of genotypes for routine purpose will help to establish the varietal identity, to measure the genetic relationship and to assess genetic diversity with the right choice of a technique, proper sampling procedure and judicious interpretation. These laboratory methods can provide reliable and accurate results for variety identification and genetic purity testing in a considerably short period of time. The SDS-PAGE electrophoresis test used to characterize paddy genotypes by total soluble seed protein content helps to differentiate important paddy genotypes based on number of bands, their relative mobility and intensity.

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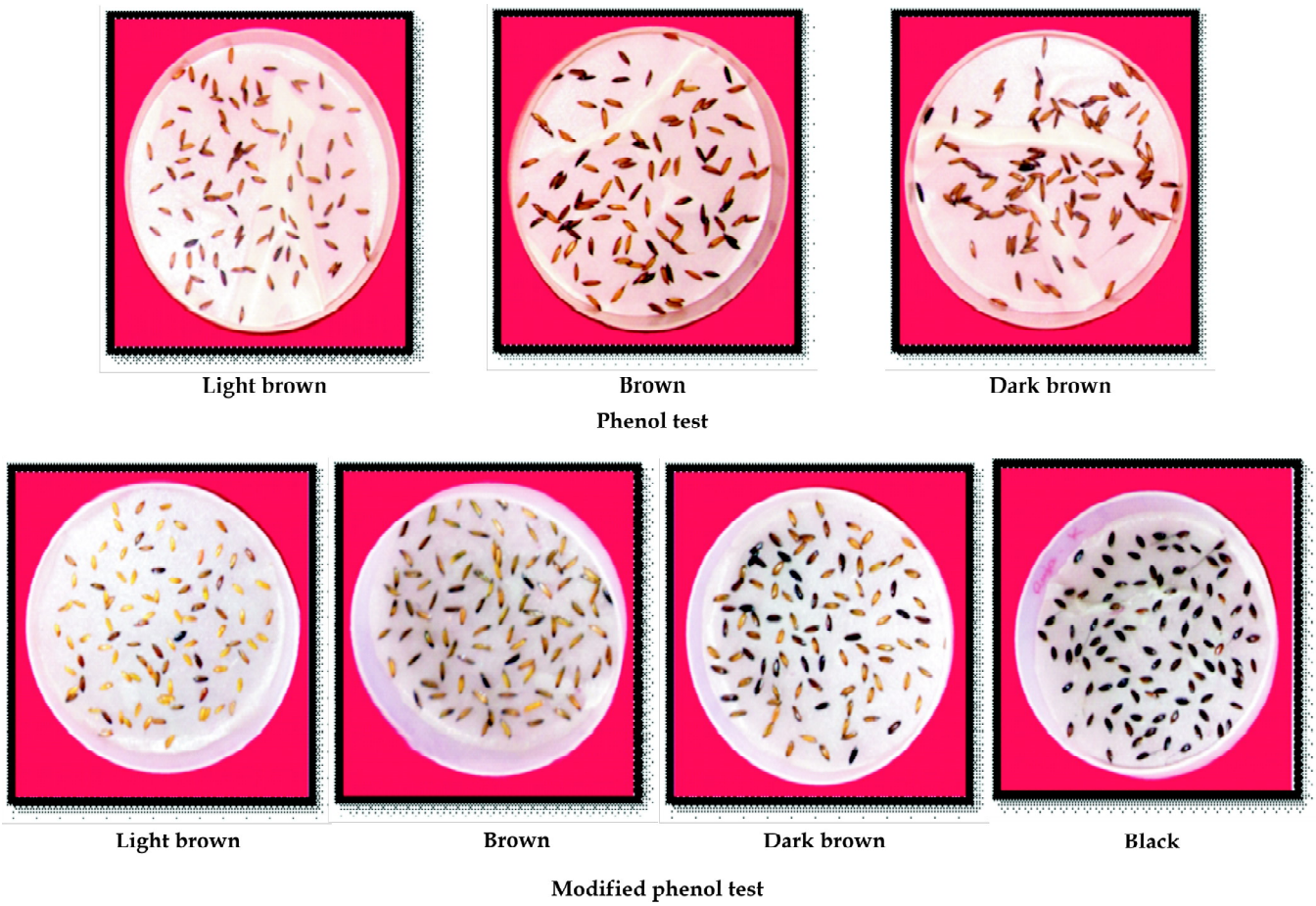


Figure 1: Phenol and modified phenol tests for identification of paddy genotypes.

MATERIAL AND METHODS

The experiments were carried out in the laboratory of Department of Seed Science and Technology, College of Agriculture, University of Agricultural Sciences, Raichur The seed material of most important different paddy genotypes *viz*, Gangavati sanna, GGV-05-01, BPT-5204, CSR-22, Gidda emergency, Gangavati mallige, Gangavati emergency, JGL-1798, Mysore mallige and Ratan sagar were obtained from Paddy breeder, AICRP on paddy, Agricultural Research Station, Gangavati for the present investigation.

Chemical test

Phenol test

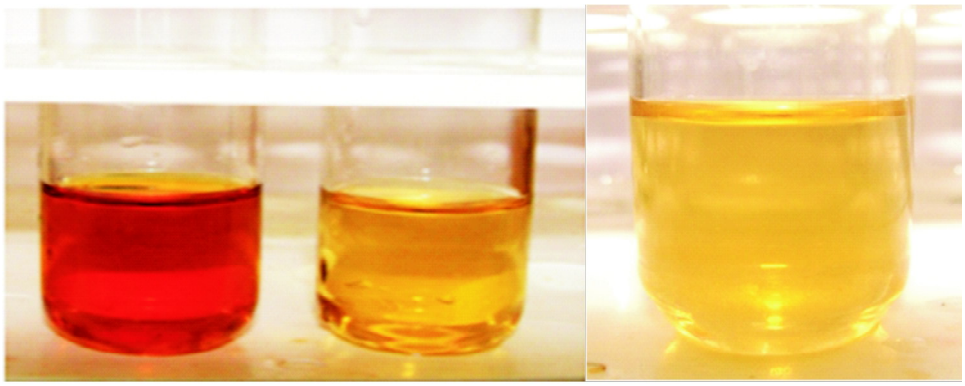
The standard phenol test for varietal purity testing as suggested by Walls (1965) was followed. Four replications of 100 seeds each were soaked in distilled water for 24 hours. The seeds were then placed in petri dishes containing filter paper moistened with 5 ml of 1% phenol solution and kept at room temperature (28°C) for 24 hours. After that, the seeds were examined and grouped into different colour classes as, no colour change, light brown, brown and dark brown (Figure 1).

Modified phenol test

As described by Banerjee and Chandra (1977), the procedure is similar to the standard phenol test except that, the seeds were soaked in a solution of 0.5% $CuSO_4$ instead of soaking the seeds in distilled water. The seeds were examined and grouped into five distinct groups namely, no colour change, light brown, brown, dark brown and black (Figure 1).

Ferrous sulphate ($FeSO_4$) test

The ferrous sulphate test as described by Gupta and Agarwal (1988) was followed. Four replications of 100 seeds were soaked in one per cent ferrous



Light brown Yellow Yellow

KOH test

Figure 2: NaOH and KOH tests for identification of paddy genotypes Gidda emergency, BPT-5204.

sulphate solution and kept in an incubator for two hours and the distinct colour groups were recorded as, brown spots, grey spots and grey streaks.

Potassium hydroxide (KOH) test

The test procedure as suggested by Mckee (1973), the seeds (1 g) of genotypes were washed in distilled water and then soaked in 10 Ml of 6% potassium hydroxide solution for one hour in test tube at an ambient temperature. The solution was decanted and used for visual observation. Based on the change in colour of the solution, the genotypes were grouped as, light yellow, light brown, brown and dark brown (Figure 2).

Sodium hydroxide (NaOH) test

Four replications of fifty seeds each were soaked in 3% NaOH solution for 3 hours and thereafter the change in colour of the solution was observed (Agrawal, 1987). Based on intensity of colour reaction, the genotypes were classified into three groups *viz.*, no colour change, light yellow and wine red (Figure 2)

Peroxidase test

It was studied as per the procedure given by Buttery and Buzzell (1968). Ten seed coats were removed and placed separately in the test tube, with three replications for all the varieties and added 10 drops of 0.5 per cent Guaiacol solution into test tube, after ten minutes one drop of 0.1 per cent solution of hydrogen peroxide (H_2O_2) was added and the reactions were noted exactly after sixty seconds. The colouration due to peroxidase activity was observed to group the varieties as, brown colour solution and colourless solution.

Seed Composition Estimation

Moisture content (%)

Five grams of powdered seed material for each treatment were taken for determining the moisture content using low constant temperature oven method. The powdered seed material was placed in a weighed metal cup and after removing the lid, moisture cups were placed in hot air oven maintained at 103 ± 2°C for 16 ± 1 h and the contents were allowed to dry. Then, the contents were weighed in an electronic balance along with metal cup and lid. The moisture content was worked out using the following formula and expressed as percentage (ISTA, 1999).

$$\text{Moisture content (\%)} = \frac{M_2 - M_3}{M_2 - M_1} \times 100$$

Where,

M_1 : Weight of the metal cup alone

M_2 : Weight of the metal cup + sample before drying

M_3 : Weight of the metal cup + sample after drying

Protein content (%)

The five gram seeds of different genotypes were weighed and powdered separately to assess protein content in the sample. Total nitrogen in powdered

sample was determined by employing the Kjeldahl's method described by Jackson (1973). Percentage of total protein was obtained by multiplying the per cent N by 6.25 factor.

Starch (%)

Hundred mg of powdered seed sample of different genotypes was taken separately into the centrifuge tube, then 5-10 ml of 80 per cent ethanol was added and the tube was placed in a water bath at 80-85°C for 5-10 min. Further centrifuge it for 10 min at 3000 rpm, decant and save the supernatant, ethanol extraction were repeated thrice and save the supernatant every time. To the residues, add 3 ml of distilled water fallowed by 6.5 ml of 52 per cent perchloric acid. Stir the content for 5 min and then occasionally for the next 15 min. Add little water and centrifuge at 3000 rpm for 5 min. Decant the supernatant into a 100 ml volumetric flask. Repeat the extraction with perchloric acid thrice, increasing the time from 15 to 25 and finally to 30 min and save the supernatant. Make up the volume of the pooled supernatants to 100 ml with distilled water. Neutralize the supernatant, make further dilutions, if necessary and estimate the glucose content of the hydrolysate by any one of the method for the estimation of reducing sugars.

Oil content (%)

The oil content of each genotype was determined with the help of NMR (Nuclear Magnetic resonance spectrometer) and the genotypes were grouped as low, and high oil content type.

Biochemical Test

Electrical conductivity (dSm^{-1})

Five grams of seeds in three replications were soaked in acetone for half a minute and thoroughly washed in distilled water for three times. Then the seeds were soaked in 25 ml distilled water and kept in an incubator maintained at 25°C ± 1°C for twelve hours. The seed leachate was collected and the volume was made up to 25 ml by adding distilled water. The electrical conductivity of the seed leachate was measured in the digital conductivity bridge (ELICO) with a cell constant 1.0 and the mean values were expressed in desi Simons per meter (dSm^{-1}) (Jackson, 1973).

Dehydrogenase activity (OD Value)

The dehydrogenase activity was measured using the method followed by Shenoy *et al.* (1990). Representative seeds (25) from each genotypes were taken and preconditioned by soaking in water for overnight at room temperature. Seeds were taken at random and the embryos were excised. The embryos were steeped in 0.25 per cent solution of 2, 3, 5-triphenyl tetrazolium chloride solution and kept in dark for two hours at 40°C for staining. The stained seeds were thoroughly washed with water and then soaked in five ml of 2 methoxy ethanol (methyl cellosolve) and kept overnight for extracting the red colour formazan. The intensity of red colour was measured using ELICO UV-VI Spectrophotometer (model SL-159) using blue filter (470 nm) and methyl cellosolve as the blank. The OD value obtained was reported as dehydrogenase activity.

Alpha amylase activity (mm)

The α-amylase activity was analyzed as per the method suggested by Simpson and Naylor (1962). Two gram of agar shreds and one gram of potato starch was mixed together in water to form paste and the volume was made up to 100 ml with distilled water. The homogenous solution of agar-starch mixture after boiling was poured into sterilized Petri-dishes and allowed to settle in the form of gel after cooling. The pre-soaked (for 8 hours) and half cut seeds (with their half endosperm and embryo portion intact) were placed in the petri-dishes in such a way that the endospermic part remained in contact with agar-starch gel. The petri-dishes were closed and kept in dark at 30^0C. After 24 hours the Petri-dishes were uniformly smeared with potassium iodide solution (0.44 g of iodine crystal + 20.008 g potassium iodide in 500 ml distilled water) and excess solution was drained off after few minutes. The diameter of halo (clear) zone formed around the seed was measured in mm and reported as α-amylase activity.

Electrophoretic analysis of total soluble protein content

Sodium Dodecyl Sulphate Polyacrylamide gel electrophoresis (SDS-PAGE) of total soluble seed protein was carried out by 10 per cent polyacrylamide gels according to the method of Laemmali (1970) with slight modification. The electrophoresis was done in vertical slab gels.

Table 1
Grouping of paddy genotypes based on seed characteristics

Genotypes	*Seed length (mm)*	*Group*	*Seed width (mm)*	*Group*	*Seed length/ width ratio*	*Group*
Gangavati mallige	9	Long	2.3	Medium	3.9	Elongated
Mysore mallige	7.6	Medium	2.4	Medium	3.1	Elongated
GGV-05-01	7.5	Medium	2.1	Narrow	3.5	Elongated
Gangavati sanna	7.3	Short	1.9	Narrow	4.0	Elongated
Gidda emergency	7.6	Medium	2.4	Medium	3.1	Elongated
Gangavati emergency	7.3	Short	2.5	Medium	2.9	Semi long
CSR-22	9.2	Long	2.4	Medium	3.8	Elongated
BPT-5204	7.4	Short	2.2	Narrow	3.3	Elongated
JGL-1798	7.2	Short	2.1	Narrow	3.4	Elongated
Ratan sagar	7.2	Short	2.1	Narrow	3.4	Elongated

Seed length			*Seed width*			*Seed length/width ratio*		
Short	:	< 7.5 mm	Narrow	:	1.9-2.2 mm	Spherical	:	< 2.0
Medium	:	7.5-9.0 mm	Medium	:	2.3-2.8 mm	Semi spherical	:	2.0-2.4
Long	:	9.0-10.0 mm	Broad	:	> 2.8 mm	Semi long	:	2.4-3.0
Very long	:	> 10 mm				Elongated	:	> 3

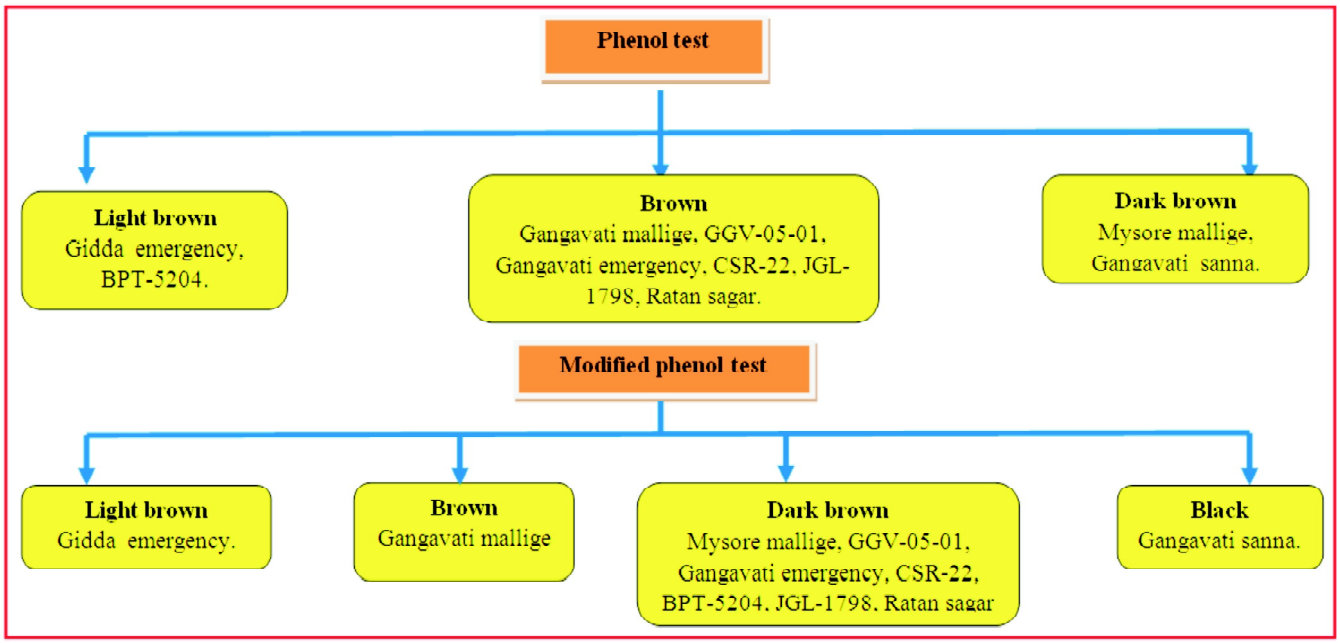


Figure 3: Identification key for paddy genotypes based on chemical test.

RESULTS AND DISCUSSION

Grouping of Paddy Genotypes Based on Chemical Test

Varietal identification by morphological characters is laborious, time consuming, tedious, cumbersome and costly affair. A number of chemical tests have been developed for varietal identification such as Phenol test, Modified phenol test, Peroxidase test, Potassium hydroxide test, Sodium hydroxide, Ferrous sulphate test. These chemical tests are very quick, easy and reproducible (Ashwani Kumar *et al.*, 1995). Very often these tests provide supportive evidence for morphological evaluation

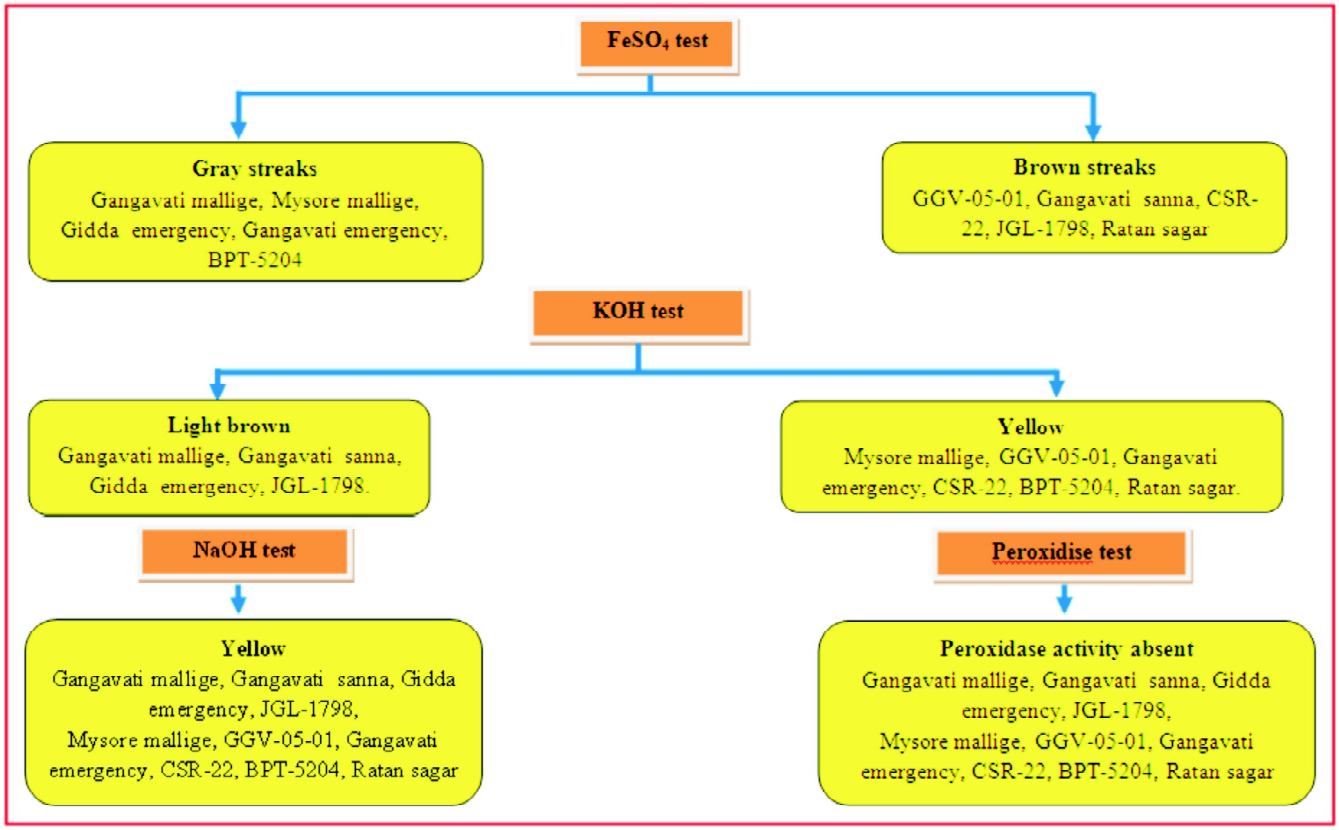


Figure 4: Identification key for paddy genotypes based on chemical test.

of seeds (Vanderburg and Vanzwol, 1991) and varietal identification keys were prepared (Figure 3 and 4).

The genotypes were grouped on the basis of the reaction of seeds to the various chemical tests (Table 1).

On the Basis of seed colouration with phenol, the paddy genotypes were grouped into three categories (light brown, brown and dark brown), two genotypes (Gidda emergency and BPT-5204), Showed light brown, six genotypes (Gangavati mallige, GGV-05-01, Gangavati emergency, CSR-22, JGL-1798 and Ratan sagar) showed brown and two genotypes (Mysore mallige and Gangavati sanna) showed dark brown.

Seed colouration with phenol is one of the important qualitative characteristic which is not affected by environmental condition. The result of phenol test is usually distinct and easily interpreted. Walls (1965) reported that the phenol colour reaction depends on the quality and quantity of oxidase enzymes present in seeds.

While on the basis of modified phenol test with $CuSO_4$, the genotypes were classified into four groups (no colour change, light brown, brown and black). One genotype (Gidda emergency) showed light brown, one genotype (Gangavati mallige) showed brown, seven genotypes (Mysore mallige, GGV-05-01, Gangavati emergency, CSR-22, BPT-5204, JGL-1798 and Ratan sagar) showed dark brown and one genotype (Gangavati sanna) showed black. Banerjee and Chandra (1977) reported Cu^{++} ions act as catalysts which increased the enzymatic process thereby enhancing the resolution of phenol colour reaction.

In ferrous sulphate test, the genotypes were grouped into two group (Grey streak and Brown streaks). Five genotypes (Gangavati mallige, Mysore mallige, Gidda emergency, Gangavati emergency and BPT-5204) showed grey streaks and remaining five genotypes showed brown streaks (GGV-05-01, Gangavati sanna, CSR-22, JGL-1798).

The potassium hydroxide test is useful in determining the varietal difference based on the chemical reaction. On the basis of colour reaction

Table 2
Grouping of paddy genotypes based on seed composition

Genotypes	*Protein content (%)*	*Groups*	*Starch content (%)*	*Groups*	*Oil content(%)*	*Groups*
Gangavati mallige	7.40	Low	72.17	Low	1.73	High
Mysore mallige	7.90	High	71.50	Low	1.23	Low
GGV-05-01	6.60	Low	75.00	High	1.90	High
Gangavati sanna	7.30	Low	70.53	Low	1.50	High
Gidda emergency	8.00	High	70.07	Low	1.70	High
Gangavati emergency	6.90	Low	70.10	Low	2.07	High
CSR-22	7.90	High	78.07	High	1.10	Low
BPT-5204	7.90	High	77.13	High	1.77	High
JGL-1798	7.23	Low	72.13	Low	1.40	Low
Ratan sagar	7.70	High	79.10	Low	1.53	High
MEAN	7.48		73.58		1.59	
S.Em±	0.061		0.118		0.035	
CD (5%)	0.181		0.349		0.103	

Protein	*Starch*	*Oil content*
High : > 7.5%	High : >75%	High : > 1.5%
Low : <7.5%	Medium : 75%	Low : < 1.5%
	Low : <75%	

with KOH solution, the genotypes were categorised into yellow and light brown. Six genotypes (Mysore mallige, GGV-05-01, Gangavati emergency, CSR-22, BPT-5204 and Ratan sagar) showed yellow and four genotypes (Gangavati mallige, Gangavati sannna, Gidda emergency and JGL-1798) showed light brown. Varied colour reaction may be due to the chemical composition of seed or selective action of enzymes present which may be governed genetically.

All ten paddy genotypes showed yellow colour reaction with sodium hydroxide solution. The difference in colour reaction of seeds seems to be due to differences in genetic back ground concerning the enzyme system (Chakrabarthy and Agrawal, 1990). In our study with respect to peroxidase test peroxidase enzyme was absent.

The chemical assays will serve as a useful tool in grouping genotypes which are crop specific based ongenitic makeup and enzymatic activity. Among the various chemical assays the paddy crop responded well for phenol, modified phenol with $CuSO_4$, $FeSO_4$, KOH tests.

Grouping of Paddy Genotypes Based on Seed Composition Analysis

The moisture content varied significantly among the paddy genotypes (Table 2). The mean moisture content of the genotypes was 13.33 per cent. The moisture content of the genotypes ranged from 12.10% (Gidda emergency) to 14.53% (Ratan sagar). Highest moisture content was noticed in Ratan sagar (14.53%) followed by BPT-5204 (13.83%), CSR-22 (13.60%), Gangavati sanna (13.60%), GGV-05-01 (13.60%), Gangavati mallige (13.27%), Mysore mallige (13.13%), Gangavati emergency (12.83%), JGL-1798 (12.80%) and Gidda emergency (12.10%).

The protein content varied significantly among the paddy genotypes (Table 2). The mean protein content of the genotypes was 7.48% per cent. The protein content of the genotypes ranged from 6.60% (GGV-05-01) to 8.0% (Gidda emergency). Based on percentage of protein content, genotypes were grouped into two groups having low (< 7.5%) and high (> 7.5%).

Among ten genotypes, five genotypes had high seed protein *viz*., Gidda emergency (8.0%) followed

Table 3
Characterization of paddy genotypes based on biochemical tests

Genotypes	*Electrical conductivity (dSm^{-1})*	*Group*	*Dehydrogenase enzyme activity (OD Value)*	*Group*	*α-amylase activity (mm)*	*Group*
Gangavati mallige	0.44	Low	0.0413	High	10.53	Low
Mysore mallige	0.53	High	0.0361	Low	10.17	Low
GGV-05-01	0.63	High	0.0417	High	11.23	High
Gangavati sanna	0.42	Low	0.0408	High	10.47	Low
Gidda emergency	0.42	Low	0.0397	Low	10.07	Low
Gangavati emergency	0.64	High	0.0391	High	10.10	Low
CSR-22	0.56	High	0.0400	High	11.43	High
BPT-5204	0.46	Low	0.0425	High	11.43	High
JGL-1798	0.53	High	0.0407	High	11.03	High
Ratan sagar	0.63	High	0.0400	High	12.30	High
MEAN	0.53		0.0401		10.88	
S.Em±	0.012		0.001		0.072	
CD (5%)	0.037		0.003			

Electrical conductivity	*Dehydrogenase enzyme activity*	*α-amylase activity*
High : > 0.5 dSm^{-1}	High : > 0.04 (OD Value)	High : > 11 mm
Low : < 0.5 dSm^{-1}	Low : < 0.04 (OD Value)	Low : < 11 mm

by BPT-5204 (7.9%), CSR-22 (7.9%), Mysore mallige (7.9%), Ratan sagar (7.7%) and five genotypes had low protein content *viz*., Gangavati mallige 7.4%), Gangavati sanna (7.3%), JGL-1798 (7.23%), Gangavati emergency (6.9%) and GGV-05-01 (6.60%).

The data pertaining to starch content (%) varied in paddy genotypes is presented in (Table 5). The mean starch content of the genotypes was 73.58% per cent. The starch content of genotypes ranged from 79.07% (Ratan sagar) to 70.10% (Gidda emergency). Based on percentage of starch content, genotypes were grouped into two groups having low (< 75%) and high (> 75%) on content.

Among 10 genotypes, three genotypes had high seed starch *viz*., Ratan sagar (79.105%) followed by CSR-22 (78.02%), BPT-5204 (77.13%) and seven genotypes had low seed starch *viz*., GGV-05-01 (75.00%), Gangavati mallige (72.17%), JGL-1798 (72.13%), Mysore mallige (71.5%), Gangavati sanna (70.53%), Gangavati emergency (70.10%) Gidda emergency (70.07%).

The results regarding to oil content (%) of paddy genotypes is presented in Table 5. Oil content varied among the genotypes. The mean oil content 1.59 per cent. The oil content of genotypes ranged from 1.10% (CSR-22) to 2.07% (Gangavati emergency). Based on percentage of oil content, genotypes were grouped into two groups having low (< 1.5%) and high (> 1.5%) on content.

Among ten genotypes, seven genotypes had high seed oil content *viz*., Gangavati emergency (2.07%), GGV-05-01 (1.90%), BPT-5204 (1.77%), Gangavati mallige (1.73%), Gidda emergency (1.70%), Ratan sagar (1.53%), Gangavati sanna (1.5%) and three genotypes had low oil content *viz*., JGL -1798 (1.40%), Mysore mallige (1.23%) and CSR-22 (1.10%).

The highest protein content was noticed in Mysore mallige, CSR-22, BPT-5204 (7.9%) and lowest in GGV-05-01 (6.60%), with the mean of 7.48%. Based on this the genotypes were grouped as low protein (< 7.5%) with five genotypes (Gangavati mallige, GGV-05-01, Gangavati sanna,

Table 4
Intensity and relative mobility of total soluble seed proteins of paddy genotypes

R_m	Genotypes									
	Gangavati mallige		Mysore mallige		GGV–05–01		Gangavati sanna		Gidda emergency	
Gangavati emergency	CSR–22	BPT–5204	JGL –1798	Ratan sagar						
0.1	+++	+++	++	+++	+++	+++	+++	–	–	–
0.16	–	–	–	–	–	–	+++	+++	+++	+++
0.18	–	–	++	–	–	++	–	–	–	–
0.2	++	++	–	++	+++	+	–	–	–	–
0.22	–	–	–	–	–	–		++	++	++
0.24				–	–	–	–	+	–	–
0.26	–	–	–	–	–	–		+	–	–
0.28	++	–	–	–	++	–	–	–	++	–
0.3	–	++	++	++	–	–	–	–	–	–
0.32	–	–	–	–	–	–	–	–	++	–
0.36	–	–	–	–	+++	+++	–	–	–	–
0.4	–	+	–	–	–	–	–	–	–	–
0.6	–	–	–	–	–	–		++	++	–

– : Bands absent
\+ : Light intense band
++ : Medium intense band
+++ : Dark intense band

Gangavati emergency and JGL-1798), high protein (> 7.5) with five genotypes (Mysore mallige, Gidda emergency, CSR-22, BPT-5204 and Ratan sagar).

The highest starch content was observed in CSR-22 (78.07%) and lowest in Gidda emergency (70.07%), with the mean of 73.58%. Based on this, the genotypes were grouped as low starch (< 75%) with seven genotypes (Gangavati mallige, Gangavati sanna, Gangavati emergency, JGL-1798, Mysore mallige, Gidda emergency and Ratan sagar) and high starch (> 75%) with three genotypes (GGV-05-01, CSR-22 and BPT-5204).

The highest oil content was noticed in Government emergency (2.07%) and lowest in CSR-22 (1.10%), with the mean of 1.59%. Based on this, the genotypes were grouped as low oil content (1.5%) with three genotypes (Mysore mallige, CSR-22, JGL-1798) and high oil content (> 1.5%) with seven genotypes (Gangavati mallige, Gangavati sanna, Gangavati emergency, Gidda emergency and Ratan sagar, GGV-05-01, and BPT-5204). Similar results were earlier reported by Luan and Han (1990) in groundnut, Shadakshri *et al*. (1995) in sesame. The variation in oil content might be a genetic factor. Weiss (1971) reported that two to seven polygenes were involved in the inheritance of oil content and varietal identification keys were prepared (Table 3).

Grouping of Paddy Genotypes Through Biochemical Analysis

The results pertaining to electrical conductivity are presented in Table 6. The electrical conductivity was ranged from 0.42 dSm^{-1} to 0.63 dSm^{-1} with mean 0.53 dSm^{-1} per cent. Genotypes were grouped into two groups having low (< 0.5 dSm^{-1}) and high (> 0.5 dSm^{-1}) on content.

Among ten genotypes, four genotypes had low electrical conductivity *viz*., Gangavati sanna (0.42 dSm^{-1}), Gidda emergency (0.42 dSm^{-1}), Gangavati mallige (0.44 dSm^{-1}), BPT-5204 (0.46 dSm^{-1}) and six genotypes had high electrical conductivity *viz*., Mysore mallige (0.53 dSm^{-1}), JGL-1798 (0.53 dSm^{-1}), CSR-22 (0.56 dSm^{-1}), GGV-05-01 (0.63 dSm^{-1}), Ratan sagar (0.63 dSm^{-1}) and Gangavati emergency (0.64 dSm^{-1}).

The results pertaining to dehydrogenase enzyme activity presented in Table 6. The Dehydrogenase enzyme activity was ranged from 0.0361-0.0425 with mean 0.0401. Genotypes were grouped into two groups having low (< 0.04) and high (> 0.04).

Among 10 genotypes, eight genotypes had high dehydrogenase enzyme activity *viz.*, Gangavati mallige (0.0413), GGV-05-01 (0.0417), Gangavati sanna (0.0408), Gangavati emergency (0.0391), CSR-22 (0.0400), BPT-5204 (0.0425), JGL-1798 (0.0407), Ratan sagar (0.0400) and two genotypes had low dehydrogenase enzyme activity *viz.*, Gidda emergency (0.0397) and Mysore mallige (0.0361).

The results regarding α-amylase activity are presented in the (Table 6 and Plate 6). The α-amylase activity was ranged from 10.07 mm to 12.30 mm with mean 10.88 mm. The genotypes were grouped into two groups having low (< 11) and high (> 11)

Among ten genotypes, five genotypes had high α-amylase activity *viz.*, GGV-05-01 (11.23 mm), CSR-22 (11.43 mm), BPT-5204 (11.43 mm), JGL-1798 (11.03 mm), Ratan sagar (12.30 mm) and another five genotypes have low α-amylase activity namely Gangavati mallige (10.53 mm), Mysore mallige (10.17 mm), Gangavati sanna (10.47 mm), Gidda emergency (10.07 mm) and Gangavati emergency (10.10 mm).

The total soluble proteins from single paddy seed were extracted and separated by SDS-PAGE and attempted to distinguish cultivar based on the electrophoratic banding pattern method. For the cultivar identification, the presence or absence of specific band, as well as band intensity were taken as the criteria for characterization of paddy genotypes. (Table 4 and Plate 7).

The Rm value for the paddy genotypes was ranged from 0.1 to 0.6. the cultivars differed in number of bands, their relative mobility and intensity. By using SDA-PAGE, the total soluble seed protein could be fractionated into 13 bands, which should heterogeneity among the different cultivars. BPT-5204 and JGL-1798 exhibited maximum number of bands (5) followed by Gidda emergency and Gangavati emergency, least number of bands in Ratan sagar (2).

The band no. 1 (Rm: 0.1) were observed in seven genotypes (Gangavati mallige, Mysore mallige, GGV-05-01, Gangavati sanna, Gidda emergency, Gangavati emergency, CSR-22), in that GGV-05-01 had medium intense band and remaining genotypes having dark intense band. The band no. 2 (Rm: 0.16) were observed in four genotypes (CSR-22, BPT-5204, JGL -1798 and Ratan sagar) having dark intense band. Band no. 3 (Rm: 0.18) were observed only in two genotypes (GGV-05-01 and Gangavati emergency) having medium intense band.

The band no. 4 (Rm: 0.2) were observed in five genotypes (Gangavati mallige, Mysore mallige, Gangavati sanna, Gidda emergency and Gangavati emergency) in that one (Gidda emergency) genotype having dark intense band, three genotypes (Gangavati mallige, Mysore mallige and Gangavati sanna) having medium intense band and one genotype having (Government emergency) light intense band. Band no. 5 (Rm: 0.22) were observed in three genotypes (BPT-5204, JGL -1798 and Ratan sagar) having medium intense band. The band 6 (Rm: 0.24) were observed in only one genotype (BPT-5204) having light intense band. The band no. 7 (Rm: 0.26) were observed in one genotype (BPT-5204) having light intense band. The band no. 8 (Rm: 0.28) were observed in three genotypes (Gangavati mallige, Gidda emergency and JGL-1798) having medium intense band.

Band no. 9 (Rm: 0.3) were observed in three genotypes (Mysore mallige, GGV-05-01 and Gangavati sanna) having medium intense band. The band no. 10 (Rm: 0.32) were observed in one genotype (JGL -1798) having medium intense band. The band no. 11 (Rm: 0.36) were observed in two genotypes (Gidda emergency and Gangavati emergency) having dark intense band.

Band no 12 (Rm: 0.4) were observed in one genotype (Mysore mallige) having light intense band whereas band no. 13 (Rm: 0.6) were observed in two genotype (BPT-5204 and JGL -1798) having medium intense genotype.

The highest electrical conductivity was recorded in Government emergency (0.64 dSm^{-1}) and lowest in Gangavati sanna, Gidda emergency (0.42 dSm^{-1}), with the mean of 0.53 dSm^{-1}). Based on this, the genotypes were grouped as low electrical

conductivity (< 0.5 dSm^{-1}) with four genotypes (Gangavati mallige, Gangavati sanna, Gidda emergency and BPT-5204) and high electrical conductivity (> 0.5 dSm^{-1}) with six genotypes (Mysore mallige, GGV-05-01, Gangavati emergency, CSR-22, JGL-1798 and Ratan sagar). Higher seed leachate due to controlled deterioration at higher moisture content was noticed as evident from electrical conductivity test. This might due to increase in the membrane damage when controlled deterioration was done at higher moisture contents.

The highest Dehydrogenase enzyme activity was observed in BPT-5204 (0.0425) and lowest in Mysore mallige (0.0361), with the mean of 0.0401. Based on this, the genotypes were grouped as low dehydrogenase enzyme activity (< 0.04) with two genotypes (Mysore mallige, gidda emergency) and high dehydrogenase enzyme activity (> 0.04) with eight genotypes (Gangavati mallige, GGV-05-01, Gangavati sanna, Gangavati emergency, CSR-22, BPT-5204, JGL-1798 and Ratn sagar). Dehydrogenase enzyme which exists in mitochondria and necessary for respiratory process indicate the level of seed viability and vigour (ISTA, 2013).

The highest α-amylase activity was recorded in CSR-22 and BPT-5204 (11.43 mm) and lowest in Gidda emergency (10.07 mm), with the mean of 10.88 mm. Based on this, the genotypes were grouped as low α-amylase activity (< 11mm) with five genotypes (Gangavati mallige, Mysore mallige, Gangavati sanna, Gidda emergency and Gangavati emergency) and high α-amylase activity (> 11mm) with five genotypes (GGV-05-01, CSR-22, BPT-5204, JGL-1798 and Ratan sagar) and varietal identification keys were prepared (Figure 7)

The electrophoresis of seed storage proteins is a method to investigate genetic variation and to classify plant varieties. Seed proteins are not sensitive to environmental fluctuations and its banding pattern is very stable which advocate for cultivar identification purpose in crop. It has been widely suggested that such banding patterns would be an important supplemental method for cultivar identification, particularly when there are legal disputes over the identity of the cultivars or when cultivars are to be protected under Intellectual Property Rights.

Seed storage proteins are encoded by multigenic loci and the production of a single locus comprises several electrophoretically separable bands. The procedure had been standardized in many crops species for different proteins fractions like albumin, globulin and glutelin. In paddy electrophoresis of seeds storage proteins had showed promising results in varietal characterization.

In the present study, an attempt was made to characterize paddy genotypes based on total soluble seed proteins separated by SDS-PAGE. A wide variation was observed in the pattern of protein bands of different genotypes and they differed in the number of bands, their relative mobility and intensity. The proteins separated on 15 per cent acrylamide gel could be distinguished and grouped based on the relative mobility

The highest no. of bands (5) were observed in BPT-5204 and JGL-1798 followed by Gidda emergency (4) and Government emergency (4), whereas Ratan sagar exhibited least no. of bands (2). The cultivar could not be characterized based on no. bands, but they could be differentiated clearly by their banding intensity and their relative mobility.

The genotypes *viz*., Gangavati mallige, Mysore mallige, GGV-05-01, Gangavati sanna, Gidda emergency, Gangavati emergency and CSR-22 similar banding pattern for banding no. 1 but band no. 2, 5, 6, 7, 12 and 13 were absent in all these genotypes except CSR-22 and Mysore mallige which showed unique banding pattern at R_m 0.16 (band no. 2) and R_m 0.4 (band no. 12), similarly BPT-5204, JGL-1798 and showed similar banding pattern at R_m 0.16 (band no. 2) and R_m 0.22 (band no. 5) which were distinct from rest of the cultivars.

The genotypes, GGV-05-01 and Gangavati emergency exhibited medium intensity band at R_m 0.18 (band no. 3) which were absent in all the genotypes, however band no. 4 were observed only in Gangavati mallige, Mysore mallige, Gangavati sanna and Gidda emergency. Hence, this is useful for cultivar identification. Cultivar BPT-5204 was distinct from all other genotypes by presence of band no. 6 and 7. Whereas, JGL-1798 showed

medium intensity band (band no. 10) at R_m 0.32. Hence, this band region could be useful in identification of these cultivar. Gidda emergency and Gangavati emergency exhibited band at R_m 0.36 (band no. 4) and BPT-5204 and JGL-1798 showed band at R_m 0.6 (band no. 13) which were distinct from other cultivar based on presence and intensity of band. Hence, this region could be useful to identify these cultivars (Figure 8).

The usefulness of SDS-PAGE technique for establishing identity and distinctness was reported in paddy genotypes by (Rohini Devi, 2000 and Anitalakshmi *et al.* 2004). Thus, SDS-PAGE analysis of protein can be advocated for varietal identification of paddy genotypes.

SUMMARY AND CONCLUSION

The genotypes were grouped based on the variation in protein content in to low (< 7.5%) with five genotypes (Gidda emergency, BPT-5204, CSR-22, Mysore mallige, Ratan sagar) and high (> 7.5%) with five genotypes (Gangavati mallige, Gangavati sanna, JGL-1798, Gangavati emergency and GGV-05-01). Based on variation in the starch content, the genotypes were grouped into low (< 75%) with seven genotypes (GGV-05-01, Gangavati mallige, JGL-1798, Mysore mallige, Gangavati sanna, Gangavati emergency and Gidda emergencey) and high (> 75%) with three genotypes (Ratan sagar, CSR-22 and BPT-5204). Based on variation in the oil content, the genotypes were grouped into low (< 1.5%) with three genotypes (JGL -1798, Mysore mallige and CSR-22), and high (> 1.5%) with seven genotypes (Gangavati emergency, GGV-05-01, BPT-5204, Gangavati mallige, Gidda emergency, Ratan sagar and Gangavati sanna).

Based on electrical conductivity the genotypes were grouped into, low (<0.5 $dSm^{-1)}$ with four genotypes (Gangavati sanna, Gidda emergency, Gangavati mallige, BPT-5204) and high (> 0.5 dSm^{-1}) with six genotypes (Mysore mallige, JGL-1798, CSR-22, GGV-05-01, Ratan sagar and Gangavati emergency). Based on dehydrogenase enzyme activity the genotypes were grouped into, low (< 0.04 OD Value) with two genotypes (Gidda emergency and Mysore mallige) and high (> 0.04 OD Value) with eight genotypes (Gangavati mallige, GGV-05-01, Gangavati sanna, Gangavati emergency, CSR-22, BPT-5204, JGL-1798, Ratan sagar). Based on α-amylase activity the genotypes were grouped into low (< 11 mm) with five genotypes (Gangavati mallige, Mysore mallige, Gangavati sanna, Gidda emergency and Gangavati emergency) and high (> 11 mm) with five genotypes (GGV-05-01, CSR-22, BPT-5204, JGL-1798, Ratan sagar).

A wide variation was observed in the pattern of protein bands of different genotypes and they differed in the number of bands, their relative mobility and intensity. The R_m value ranged from 0.1 to 0.6.

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