Int.J.Curr.Microbiol.App.Sci (2021) *10*(01): 1480-1496

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 10 Number 01 (2021)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2021.1001.176

Characterization of Rice Genotypes Based on Physical, Chemical and Molecular Methods

E. Sudeep Kumar[1*], S. N. Vasudevan[2], N. M. Shakuntala[1], S. R. Doddagoudar[1], B. G. MasthanReddy[3] and K. Mahantashivayogayya[3]

[1]Department of Seed Science & Techonology, [3]ARS Gangavath, UAS Raichur, India
[2]Director of Research, ZARS Mandya, UAS Bengaluru, India

**Corresponding author*

Keywords

Biovis seed image analyzer, Phenol test, $FeSO_4$ test, KOH test, NaOH test, Peroxidase test, SSR markers

Article Info

Accepted:
12 December 2020
Available Online:
10 January 2021

A B S T R A C T

The present investigation was carried out to know the response and characterization of rice genotypes by physical, chemical and molecular methods. Seeds of 17 rice genotypes were analysed for seed dimension using Biovis seed image analyzer. The mean performance for seed length varied from 9.28 mm in genotype RYC-230 to 11.50 mm in genotypeMT-4420 and seed width varied from 2.64 mm in genotype IET-18299 to 4.85 mm in genotype MT-4420. The mean seed length/seed width ratio ranged from 2-37 to 3-53 among the genotypes. Rice genotypes also well responded to chemical tests such as phenol test, $FeSO_4$ test, NaOH test and KOH test and helpful in grouping of the rice genotypes. Of the 22 single sequence repeats (SSR) markers used in the study, all markers were able to distinguish rice genotypes and may be utilized to develop new varieties during crop improvement programme.

Introduction

Rice (*Oryza sativa* L.) is the primary cereal crop grown in many countries especially in Asia. The increasing population rate challenges the world's food requirements and an area expansion under rice likely to decline nowadays because of urbanization, climate change and high-value agriculture. Hence, more stress has been given for rice crop improvement since ages, which has resulted in the release of a large number of varieties with improved yield or tolerance to biotic and abiotic stresses. As India is a signatory for the WTO, and The Protection of Plant Varieties and Farmer's Rights (PPV & FR) Act, 2001 came into existence, varietal characterization and identification became more significant in the present scenario because India is having rich gene pool. Many countries including International Seed Testing Association (ISTA), Switzerland have been conducting elaborate research for developing standard chemical tests, physiological tests and use of

various biomolecular markers for varietal identification in crops.

The shape, size and colour of seeds are normally employed to identify rice varieties. Use of computer-based image analysis is a good alternative to visual identification. Hiremath (2013) grouped rice genotypes based on seed length and seed width using Marvin seed image analyser. Similarly, Maruthi (2016) classified 180 paddy genotypes based on seed length and seed width using Biovis seed image analyser. Various studies on the characterization of cultivars confirmed that the response of seed and seedlings to various chemical tests *viz*., phenol test, modified phenol test, ferrous sulphate test, potassium hydroxide test offer a wider variability and can be used in the characterization of genotypes.

The simple, reliable and quick chemical tests can be used for varietal identification in rice crop (Vijayalakshmi and Vijay, 2009).The chemical tests gave the stable results and could be effectively used for cultivar differentiation and determining the varietal purity of rice for routine testing in seed testing laboratories as some of the cultivars showed a distinct response to these phenol test (Anitalakshmi *et al.,* 2014). Masuthi *et al.,* (2015) used 32 paddy genotypes for phenol test, out of which 12 genotypes showed no colour change, 15 genotypes were light brown, 8 genotypes were brown and 6 genotypes were dark brown in colour. Saharan (1991) classified 33 genotypes of rice into four groups *viz*., brown spot, brown streaks, grey spot and grey streaks kernels by using 1.5 per cent ferrous sulphate solution. Similar classification was also done by Hiremath (2013) in rice genotypes and Raju *et al*., (2017) in rice hybrid with $FeSO_4$ test. Among the ten rice genotypes, four genotypes (Gangavatimallige, Gangavatisanna, Gidda emergency and JGL-1798) showed light brown and six genotypes (Mysore mallige, GGV-05-01, Gangavati emergency, CSR-22, BPT-5204 and Ratansagar) showed yellow colour reaction with KOH test (Hiremath, 2013). The similar grouping was done by Masuthi *et al.,* (2015) in rice genotypes and Raju *et al.,* (2017) in rice hybrids by using KOH test.

Therefore, an investigation was carried with the seventeen rice genotypes to characterize and to know the response of rice genotypes to various physical, physiological, chemical tests and by molecular markers, which helps in the determination of cultivar purity in rice.

Materials and Methods

The present investigation was carried out during the year 2016-17 in the Department of Seed Science and Technology, College of Agriculture, Raichur. Seventeen rice genotypes (G_1:RYC-230, G_2:GNV-1405, G_3:GNV-1089, G_4:GNV-1301, G_5:GNV-05-01, G_6:GNV-1109, G_7:MT-4253, G_8:IET-22066, G_9:MT-4021, G_{10}:BPT-5204, G_{11}:IET-18299, G_{12}:MT-4420, G_{13}:MTU-1010, G_{14}:MT-4541, G_{15}:IET-19251, G_{16}:MAS-26 and G_{17}:MAS-946-1)were used for image analysis, chemical tests and molecular studies.

Physical method by Biovis seed image analyzer

The seed image analyzer provides more information than a traditional counting device. It works fast, noiseless and easily operated. The features of varieties may be stored in a pre-configured, able to learn database for recognizing and identifying main seeds, foreign seeds and non-seed particles. The Biovis seed image analyzer is a device that comprises a scanner, which scans the images and captures the same with their length, width, area, perimeter and roundness with coloured images. The special Biovis

image analysis software offers a lot of possibilities, to adjust the recording of the analysis results and statistical interpretation according to the requirements and demands of the user. Thus, it is suitable for the analysis of the seeds of many crops.

Seed length

100 seeds of each genotype were measured using Biovis image analyzer. The mean value of seed length was expressed in millimetre and was grouped according to DUS test guidelines for rice as very short (< 6.0 mm), short (6.1-8.5 mm), medium (8.6-10.5 mm), long (10.6-12.5 mm) and very long (>12.5 mm)

Seed width

100 seeds of each genotype were measured using Biovis image analyzer. The mean value of seed length was expressed in millimetre and was grouped according to DUS test guidelines for rice as very narrow (< 2.0 mm), narrow (2.1-2.5 mm), medium (2.6-3.0 mm), broad (3.1-3.5 mm) and very broad (> 3.5 mm).

By chemical tests

The chemical tests find variations among the seeds and seedlings of different crop varieties. These tests do not require technical expertise or training and can be completed in a relatively short period time. The results of these tests are usually distinct, easily interpreted and help in the grouping of the genotypes.

Phenol test

The standard phenol test for varietal purity testing suggested by Walls (1965) was followed. Four replicates of 50 seeds each were soaked in distilled water for 18 h. The seeds were then placed in Petri dishes containing filter paper moistened with 4 ml of 2 per cent phenol solution and kept at room temperature (28°C). After 24 h, seeds were examined and grouped into different colour classes viz., as no colour change, light brown, brown and dark brown.

Ferrous Sulphate ($FeSO_4$) test

As described by Gupta and Agrawal (1988), fifty seeds each in four replications were soaked in 1.5 per cent FeSO4 solution for 4 h under ambient condition and later the seeds were taken out and the excess moisture was removed using blotting paper before evaluation. The seeds were examined for colour reaction and the distinct colour groups were recorded as brown spots, grey spots and grey streaks.

Potassium hydroxide (KOH) test

Seeds were soaked in 5 per cent KOH solution in 4 replications of 50 seeds each and kept at room temperature (28°C) for three hours. The colour change of the KOH solution was observed and based on the colour reaction the genotypes were divided into two groups viz., deep wine red and no staining.

Sodium hydroxide (NaOH) test

Four replications of 50 seeds each were soaked in 5 per cent NaOH solution for one hour and the change in colour of the solution was observed. Based on the colour developed, varieties were classified into three groups viz., dark yellow, light yellow and no colour.

Peroxidase (H_2O_2) 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 genotypes and added 10 drops of 0.5 per cent Guaiacol solution into the 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 present and absent of brown colour solution.

By Molecular markers

Twenty two Rice Microsatellite (RM) markers were used to study the polymorphism among the seventeen rice genotypes (Table 1.) that are screened under Direct seeded rice (DSR) method. The method followed for DNA extraction was Modified Cetyltrimethyl Ammonium Bromide (CTAB) method (Cao and Oard, 1997).

Results and Discussion

Grouping of genotypes based on seed morphology using Biovis Seed Image Analyzer

Seed length (mm)

Seeds of 17 rice genotypes were analyzed for seed dimension using Biovis seed image analyser. The mean performance for seed length varied from 9.28 mm in RYC-230 to 11.50 mm in MT- 4420 and seed width varied from 2.64 mm in IET-18299 to 4.85 mm in MT-4420 (Table 2.& 3.). The genotypes *viz*., RYC-230, GNV-1405, GNV-1089, GNV-05-01, GNV-1109, BPT-5204, IET-18299 and IET-19251 are having medium seed length and genotypes GNV-1301, MT-4253, IET-22066, MT-4021, MT-4420, MTU-1010, MT-4541, MAS-26 and MAS-946-1 possess longer seed length and none of them are very short, short and very long. Similarly, seed width in rice genotypes *viz*., RYC-230, GNV-1405, GNV-05-01, BPT-5204, IET-18299 and IET-19251 was medium while, genotype GNV-1089 was broad type and genotypes GNV-1301, GNV-1109, MT-4253, IET-22066, MT-4021, MT-4420, MTU-1010, MT-4541, MAS-26 and MAS-946-1 falls under very broad width category. None of the genotypes in seed width was a very narrow and narrow group. So also, the mean seed length/seed width ratio ranged from 2.37 to 3.53among the genotypes. Four genotypes were grouped as semi-spherical, five genotypes as semi-long and remaining eight genotypes were grouped as elongated. A similar study of image analysis on seed length and width were carried out by Paulsen et al (1989) in maize, Zayas *et al.,* (1989, 1996), Utku *et al.,* (1998) in rice, Suchowilska and Wiwari (2006) and Sainis *et al.,* (2009) in wheat. Variations based on seed size and shape and other seed parameters have also been reported by Anuradha *et al.,* (2009), Iwata *et al.,* (2010), Hiremath (2013) and Maruthi (2016) in paddy.

Identification of rice genotypes through chemical tests

Phenol test

Based on seed colouration with phenol, the rice genotypes studied were grouped into three categories viz., no colour, light brown, brown and dark brown. Three genotypes viz., G_2:GNV-1405, G_{11}:IET-18299 and G_{14}:MT-4541 showed no colour, four genotypes (G_1:RYC-230, G_4:GNV-1301, G_6:GNV-1109 and G_9:MT-4021) showed brown colour, two genotypes (G3-GNV- 1089 and G8-IET-22066) showed dark brown and eight genotypes (G_5:GNV-05-01, G_7:MT-4253, G_{10}:BPT-5204, G_{12}:MT-4420, G_{13}:MTU-1010, G_{15}:IET-19251, G_{16}:MAS-26 and G_{17}:MAS-946-1) showed light brown colour (Fig.1). Similarly, the genotypes were grouped earlier based on phenol colour reaction in rice by Meshram and Rahangdale

(1988), Wang and Shen (1992), Vanagamudi *et al.*, (1988), Jaiswal and Agarwal (1995), Bora *et al.*, (2008), Devi Singh *et al.*, (2011), Tiwari *et al.*, (2013) and Masuthi *et al.*, 2015) in rice. The present findings reveal that phenol test could be used as a simple, quick and cheap method for grouping paddy genotypes. The difference in the phenol colour reaction of hulls seems to be due to the differences in the genetic background, presumably concerning the enzyme system. Seed colouration with phenol is one of the important qualitative characteristics which is not affected by the 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. Whereas, Takahashi and Hamza (1983) reported monophenol oxidase was extremely localized in grains even though it is present in all other plant parts. Phenol colour reaction, which is an index of polyphenol oxidase activity, has been utilized to distinguish the crop varieties by earlier workers Joshi and Banerjee (1970) in wheat, Abrol and Uprety (1972) and Chauhan and Nanda (1984) in rice.

Table.1 List of SSR primers used to study the polymorphism among rice genotypes

Sl.No.	Marker	Forward primers (5'-3')	Reverse primers (3'-5')
1.	RM241	Gagccaaataagatcgctga	Tgcaagcagcagatttagtg
2.	RM257	Cagttccgagcaagagtactc	Ggatcggacgtggcatatg
3.	RM224	Atcgatcgatcttcacgagg	Tgctataaaaggcattcggg
4.	RM219	Cgtcggatgatgtaaagcct	Catatcggcattcgcctg
5.	RM252	Ttcgctgacgtgataggttg	Atgacttgatcccgagaacg
6.	RM562	Cacaacccacaaacagcaag	Cttcccccaaagttttagcc
7.	RM140	Tgcctcttccctggctcccctg	Ggcatgccgaatgaaatgcatg
8.	RM448	Tctgatcttgatgcaggcac	Tctcccgatttggacagatc
9.	RM578	Ggcgtcgtgttttctctctc	Caaaaaggaggagcagatcg
10.	RM286	Ggcttcatctttggcgac	Ccggattcacgagataaactc
11.	OSR28	Agcagctatagcttagctgg	Actgcacatgagcagagaca
12.	RM311	Tggtagtataggtactaaacat	Tcctatacacatacaaacatac
13.	RM171	Aacgcgaggacacgtacttac	Acgagatacgtacgcctttg
14.	RM452	Ctgatcgagagcgttaaggg	Gggatcaaaccacgtttctg
15.	RM13	Tccaacatggcaagagagag	Ggtggcattcgattccag
16.	RM306	Caaggtcaagaatgcaatgg	Gccactttaatcattgcatc
17.	RM5389	Tcttgcatgagagccaacac	Gctattgcgcgagattatcc
18.	RM161	Tgcagatgagaagcggcgcctc	Tgtgtcatcagacggcgctccg
19.	RM44	Acgggcaatccgaacaacc	Tcgggaaaacctaccctacc
20.	RM547	Taggttggcagacctttcg	Gtcaagatcatcctcgtagcg
21.	RM55	Ccgtcgccgtagtagagaag	Tcccggttattttaaggcg
22.	RM231	Ccagattatttcctgaggtc	Cacttgcatagttctgcattg

Table.2 Seed length, seed width and seed length/ width ratio as influenced by rice genotypes

Genotypes	Seed length (mm)			Seed width (mm)			Seed length/ width ratio		
	2015	2016	Pooled mean	2015	2016	Pooled mean	2015	2016	Pooled mean
G_1: RYC-230	9.23	9.33	9.28	2.73	2.64	2.69	3.45	3.54	3.49
G_2: GNV-1405	9.40	9.53	9.47	2.90	2.80	2.85	3.25	3.40	3.32
G_3: GNV-1089	9.97	10.10	10.03	3.47	3.35	3.41	2.88	3.01	2.95
G_4: GNV-1301	10.16	10.33	10.25	3.66	3.54	3.60	2.78	2.92	2.85
G_5: GNV-05-01	9.43	9.57	9.50	2.93	2.84	2.88	3.23	3.38	3.30
G_6: GNV-1109	10.43	10.57	10.50	3.93	3.80	3.87	2.65	2.78	2.72
G_7: MT-4253	11.33	11.47	11.40	4.83	4.67	4.75	2.35	2.46	2.40
G_8: IET-22066	10.23	10.37	10.30	3.73	3.61	3.67	2.76	2.88	2.82
G_9: MT-4021	11.37	11.40	11.38	4.87	4.70	4.79	2.34	2.43	2.38
G_{10}: BPT-5204	9.47	9.60	9.53	2.97	2.87	2.92	3.19	3.35	3.27
G_{11}: IET-18299	9.18	9.32	9.25	2.68	2.59	2.64	3.47	3.59	3.53
G_{12}: MT-4420	11.43	11.57	11.50	4.93	4.77	4.85	2.32	2.43	2.37
G_{13}: MTU-1010	11.34	11.34	11.34	4.83	4.67	4.75	2.35	2.43	2.39
G_{14}: MT-4541	10.47	10.60	10.53	3.97	3.83	3.90	2.64	2.77	2.70
G_{15}: IET-19251	9.47	9.60	9.53	2.97	2.87	2.92	3.19	3.35	3.27
G_{16}: MAS-26	10.45	10.87	10.66	3.93	3.80	3.87	2.69	2.86	2.77
G_{17}: MAS-946-1	10.33	10.47	10.40	3.83	3.71	3.77	2.78	2.82	2.80
Mean	**10.22**	**10.35**	**10.29**	**3.72**	**3.59**	**3.65**	**2.84**	**2.96**	**2.90**
S.Em±	0.23	0.24	0.22	0.23	0.07	0.12	0.14	0.07	0.04
CD @ 1%	0.89	0.91	0.84	0.89	0.28	0.44	0.55	0.27	0.16

Table.3 Grouping of rice genotypes based on seed length (mm), seed width (mm) and seed length / width ratio (mm)

Sl.no.	Groups	Number of Genotypes	Name of the Genotype
Seed length (cm)			
1.	Short (6.1-8.5 mm)	-	-
2.	Medium (8.6-10.5 mm)	8	RYC-230, GNV-1405, GNV-1089, GNV-05-01, GNV-1109, BPT-5204, IET-18299 and IET-19251
3.	Long (10.6-12.5 mm)	9	GNV-1301, MT- 4253, IET-22066, MT- 4021, MT- 4420, MTU- 1010, MT- 4541, MAS -26 and MAS-946-1
Seed width (mm)			
1.	Very narrow < 2.0 mm	-	-
2.	Narrow (2.1-2.5 mm)	-	-
3.	Medium (2.6-3.0 mm)	6	RYC-230, GNV-1405, GNV-05-01, BPT-5204, IET-18299 and IET-19251
4	Broad (3.1-3.5 mm)	1	GNV-1089
Seed length / width ratio (mm)			
1.	Semi spherical	4	MT-4253, MT-4021, MT-4420 and MTU-1010
2.	Semi long	5	GNV-1409, IET-22066, MT-4541, MAS-26 and MAS-946-1
3.	Elongated	8	RYC-230, GNV-1405, GNV-1089, GNV-1305, BPT-5204, IET-18299 and IET-19251

Table.4 Identification of rice genotypes through chemical tests

Genotypes	Phenol test	FeSO4 test	KOH test	NaOH test	Peroxidase test
G_1: RYC-230	Brown	Grey Streaks	Light Yellow	Yellow	Absent
G_2: GNV-1405	No colour	Grey Streaks	Light Yellow	Yellow	Absent
G_3: GNV-1089	Dark Brown	No streaks	Light Yellow	Yellow	Absent
G_4: GNV-1301	Brown	No streaks	Dark Brown	Yellow	Absent
G_5: GNV-05-01	Light Brown	No streaks	Light Yellow	Yellow	Absent
G_6: GNV-1109	Brown	Brown Streaks	Light Yellow	Yellow	Absent
G_7: MT-4253	Light Brown	Grey Streaks	Light Yellow	Yellow	Absent
G_8: IET-22066	Dark Brown	Brown Streaks	Light Yellow	Yellow	Absent
G_9: MT-4021	Brown	Grey streaks	Light Yellow	Yellow	Absent
G_{10}: BPT-5204	Light Brown	No streaks	Light brown	Yellow	Absent
G_{11}: IET-18299	No colour	Grey Streaks	Light brown	Yellow	Absent
G_{12}: MT-4420	Light Brown	Brown Streaks	Light Yellow	Yellow	Absent
G_{13}: MTU-1010	Light Brown	Brown Streaks	Dark Brown	Yellow	Absent
G_{14}: MT-4541	No colour	No streaks	Light Yellow	Yellow	Absent
G_{15}: IET-19251	Light Brown	Grey Streaks	Light Yellow	Yellow	Absent
G_{16}: MAS-26	Light Brown	No streaks	Light Yellow	Yellow	Absent
G_{17}: MAS-946-1	Light Brown	Brown Streaks	Light Yellow	Yellow	Absent

Phenol test: No colour, Light brown, Dark brown and Brown
$FeSO_4$test : Dark Grey streaks, Brown streaks and No streaks
KaOH test: No colour, Light Yellow, Dark Brown, Brown
NaOHtest : No Colour, Yellow Colour and Wine Red
Peoxidasetest : Present, Absent

Table.5 DNA polymorphism generated using 22 SSR primers in 17 rice genotypes

Sl. No.	SSR marker	Expected product size (bp)	Observed product size (bp)	No. of a Alleles produced	Major allele frequency	PIC Value
1	RM-241	100-170	125-140	3	0.70	0.46
2	RM-257	100-175	140-150	2	0.76	0.36
3	RM-224	150-180	155-170	3	0.53	0.61
4	RM-219	200-300	190-210	3	0.58	0.55
5	RM-252	215-254	215-220	3	0.52	0.58
6	RM-562	243	200-260	3	0.58	0.55
7	RM-140	261	240-260	3	0.41	0.65
8	RM-448	228	210-240	3	0.41	0.66
9	RM-578	280-310	300-320	3	0.52	0.50
10	RM-286	98-122	90-110	3	0.52	0.55
11	OSR-28	120-140	125-150	3	0.64	0.52
12	RM-311	179	170-190	2	0.76	0.36
13	RM-306	155	160-170	2	0.58	0.48
14	RM-5389	132	120-130	3	0.58	0.57
15	RM-161	187	180-190	2	0.94	0.11
16	RM-44	100-130	90-100	3	0.52	0.55
17	RM-547	235	230-250	3	0.82	0.30
18	RM-55	215-240	200-220	3	0.64	0.51
19	RM-231	168-185	170-190	3	0.58	0.57
20	RM-13	130-150	130-150	3	0.88	0.19
21	RM-452	105	105-110	2	0.88	0.21
22	RM-171	320-345	340-260	3	0.52	0.60
Minimum		**98**	**90**	**2**	**0.41**	**0.11**
Maximum		**345**	**260**	**3**	**0.88**	**0.66**
Mean		**-**	**-**	**2.5**	**0.6**	**0.5**

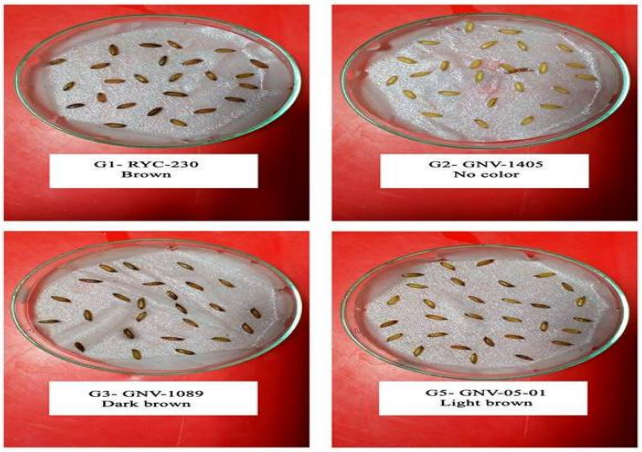

Fig. 1 Response of rice genotypes to Phenol test

Fig.2 Response of rice genotypes to $FeSO_4$ test

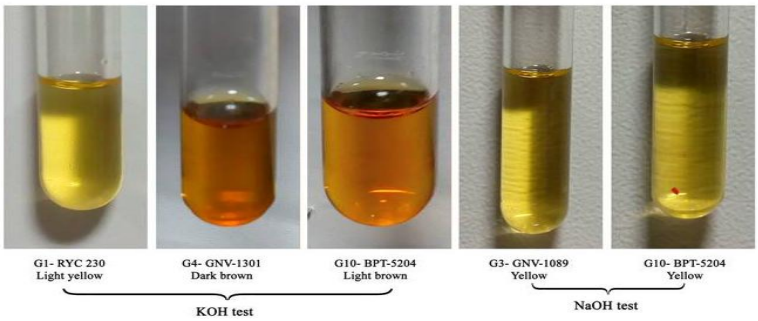

Fig. 3 Response of rice genotypes to KOH test and NaOH test

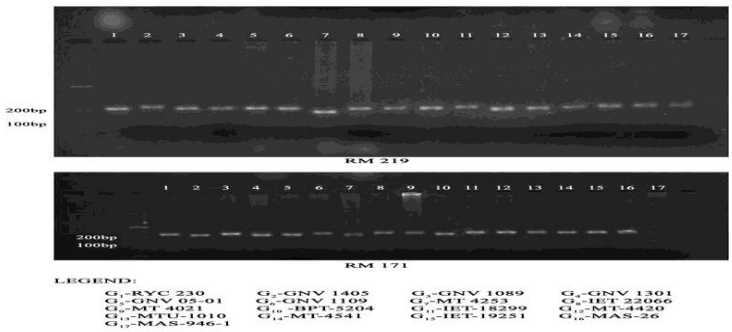

Fig. 4 SSR marker profile of rice genotypes generated by the primer RM 219 and RM 171

Ferrous sulphate ($FeSO_4$) test

In ferrous sulphate test, the genotypes were grouped into two groups (No streaks, Grey streaks and Brown streaks). Six genotypes (G_1:RYC-230, G_2:GNV-1405, G_7:MT - 4253, G_9:MT- 4021, G_{11}:IET-18299 and G_{15}:IET-19251) showed grey streaks, six genotypes (G_3:GNV-1089, G_4:GNV-1301, G_5:GNV-05-01, G_{10}:BPT-5204, G_{14}:MT-4541 and G_{16}:MAS-26) showed no streaks and five genotypes (G_6:GNV-1109, G_8:IET 22066, G_{12}:MT-4420, G_{13}:MTU-1010 and G_{17}:MAS-946-1) (Fig. 2) showed brown streaks. The similar grouping was also reported by Gupta and Agrawal (1988) in paddy, Saharan (1991) in paddy, Ponnusamy *et al.,* (2003) in cotton and Mathad *et al.,* (2019) in pigeon pea using pixel luminance test.

Potassium hydroxide (KOH) test

In the case of potassium hydroxide test, the genotypes showed a positive response to this test, as well they turned yellow colour solution. However, the intensity of colour varied among the varieties. Out of seventeen genotypes, two genotypes (G_4: GNV-1301 and G_{13}: MTU-1010) (Fig. 3) have shown dark brown colour change and the rest of the genotypes reacted to light yellow colour. Similar groupings were reported by Mckee (1973) in wheat, Rosta (1975), SambasivaRao et al (2002) in groundnut and BiradarPatil *et al.,* (2006) in safflower genotypes and Masuthi *et al.,* (2015) in rice. The varied colour reaction may be due to the chemical composition of seed or selective action of enzymes present which may be governed genetically.

Sodium hydroxide (NaOH) test

All the 17 rice genotypes showed yellow colour reaction with sodium hydroxide solution (Fig. 3). Similar results were reported by Sambasivarao *et al.,* (2002) in rice, Ponnuswamy *et al.,* (2003) in cotton and BiradarPatil (2006) in safflower genotypes, Tiwari *et al.,* (2013) in rice, Masuthi *et al.,* (2015) in rice and Mathad *et al.,* (2019) in pigeon pea. The colour reaction to sodium hydroxide solution was obtained due to the reaction of seeds to secondary metabolites (Vanderburg and Vanzwol, 1991). The difference in colour reaction of seeds seems to be due to differences in the genetic background concerning the enzyme system (Chakrabarthy and Agrawal, 1990). Potassium hydroxide and sodium hydroxide tests are useful to distinguish red seed varieties from white seed varieties if the seed coat colour of red seeded varieties vanished due to unfavourable climate condition. Vanagamudi *et al.,* (1988) reported that among 85 rice varieties, 71 varieties showed negative reaction to these chemicals and remaining varieties showed deep-wine red staining.

Peroxidase (H_2O_2) test

All the genotypes studied showed the negative reaction to peroxidase activity. Similar negativity of the reaction was also reported by Hiremath (2013) in rice genotypes. The results of peroxidase test are not conformity with previous work reported by Chakrabarthy and Agrawal (1989) in black gram, Agarwal and Pawar (1990) in soybean and Kirankumar Reddy (2004) in cotton,

Identification of rice genotypes using SSR molecular markers

In the present study, 22 SSR primers were used which produced scorable, unambiguous markers. A total of 61 alleles were produced and all are polymorphic(Fig. 4). The number of alleles detected per primer pair ranged from 2 in five primers to 3 in rest of 17 primers with an average of 2.77. The SSR

products size ranged from 90 to 340 bp. The alleles showed a high degree of polymorphism. PIC value varied significantly for all the studied SSR loci. In the present study, the level of polymorphism among the 17 genotypes was evaluated by calculating PIC values for each of the SSR loci. The PIC value ranged from 0.11 (RM161) to 0.66 (RM 448) with an average of 0.46 per marker.

Markers with PIC values of 0.5 or higher are highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate of a marker at a specific locus. This indicated that the genotypes used in the present study were more diverse due to differences in their genetic constitution. Presence of polymorphism between genotypes revealed that the presence of genetic diversity at the molecular level was high among the selected genotype which implies that, the genetic diversity is more than the morphological diversity and there is ample scope to utilize the material in the breeding programme. These results corroborate the earlier findings of Shahriar *et al.,* (2014), Kundur *et al.,* (2015) and Maruthi (2016) in rice.

Acknowledgements

This research was sponsored by UGC-NFSC Scheme, Government of India.

References

Abrol, Y. P. and Uprety, D. C., 1972.Phenol colour reaction for discolouration of paddy. Bull. Grain Tech, 10: 288-290.

Agarwal, R. L. and Pawar, A., 1990. Identification of soybean varieties based on seed and seedling characteristics. Seed Research, 18 (1): 77-81.

Anitalakshmi, V., Gowda, R., Sathisha, C. S. and Rajendra, P., 2014. Varietal response to various chemical tests for their characterization in rice. Indian Journal of Plant Sciences, 3: 2319-3824.

Anuradha, R., Balamurugan, P., Srimathi, P. and Sumathi, S., 2009. Influence of seed size on seed quality of chickpea (Cicerarietinum L.). Legume Res. Int. J., 32:133-135.

Ashwani, K., Chowdhary, R. K., Kapoor, R. L. and Dahiya, O. S., 1995.Identification of pearl millet hybrids and their parental lines using seeds and seedling characters, chemical tests and gel electrophoresis. Seed Science and Technology., 23: 21-32.

BiradarPatil, N. K., SangeetaMacha, Motagi, B. N., Vijaykumar, A. G. and Hanchinal, R. R., 2006, Characterization of safflower varieties through chemical tests. Abst.XII Nation. Seed Sem., 24-26 February, ANGRAU, Hyderabad, p.168.

Bora, R., Deka, S. D. and Sen, P., 2008. Identification of rice varieties of Assam based on grain characters and reaction to certain chemical tests. Seed Research, 36: 51-55.

Buttery, B. R. and Buzzell, R. I., 1968, Peroxidase activity in seeds of soybean varieties. Crop Sci., 8: 722-724.

Cao, D. and Oard, J. H., 1997. Pedigree and RAPD-based DNA analysis of commercial U.S. Rice cultivars. Crop Sci., 37: 1630-1635.

Chakrabarthy, S. K. and Agarwal, R. L., 1990. Identification of blackgram varieties- III: Utilization of seedling growth response to added chemical. Seed Res., 18 (1): 24-31.

Chauhan, J. S. and Nanda, J. S., 1983, Genetic variability for physic-chemical characters of rice grain in segregation *O. Sativa* L. Oryza. 20:209-215.

Devi Singh, Anuj Kumar, Navneet Kumar, Vipin Kumar, Sachin Kumar, AmalBharadwaj, PreetiSirohi and Chand, S., 2011. Comparison of traditional physic-chemical methods and

molecular marker assays for characterization of Basmati rice (*Oryzasativa* L.). African J. of Biotechnology.10(62):13390-13398.

Gupta, P. K. and Agarwal, R. L., 1988. Determination of varietal purity of paddy varieties by laboratory evaluation. Oryza. 25: 310-314.

Hiremath, M., 2013. Characterization of paddy genotypes through image analyzer. M. Sc., (Ag.) theses, UAS Raichur, Karnataka.

Iwata, H., Ebana, K., Uga, Y., Hayashi, T. and Jannink, J. L., 2010.Genomic-wide association study of grain shape among *Oryzasativa* L. germplasms based on elliptic Fourier analysis. Mol. Breed., 25:2013-215.

Jaiswal, J. P. and Agarwal, R. L., 1995. Varietal purity determinations in rice modification of the phenol test. Seed Sci. & Technol., 23: 33-42.

Joshi, M. G. and Banerjee, S. K., 1970. Genetics of phenol colour reaction in emmer wheat. Association of Official Seed Analyst, p. 28. Journal of Applied and Natural Science.6 (1): 258-261.

Kirankumar Reddy, C., 2004.Studies on laboratory techniques for identification of cotton (*Gossypium* spp.) genotypes. M.Sc. (Agri.) Thesis, Acharya N.G. Ranga Agric. Univ., Hyderabad.

Kundur, P. J., Prakash, G., Patil, B. G., Harish, C. K., Ramesh and. Shahidhar, H. E., 2015.Molecular characterization of rice (*Oryzasativa* L.) genotypes using target region amplification polymorphism (TRAP) markers in relation to grain iron content.IJAAR. 7(1):125-133.

Maruthi, J. B., 2016. Evaluation and enrichment of seed micronutrient (Fe and Zn) content through biofortification in paddy genotypes. Ph. D (Ag.) theses, UAS Raichur, Karnataka.

Masuthi, D. A., Vyakarnahal, B. S., Deshpande, V. K., Jagadeesha, R. C., Mukesh, L. C. and Khaja, R. S., 2015.Characterization of traditional aromatic rice cultivars by chemical markers. Int. J. of Advanced Res., 3: 103-107.

Mathad, R. C., Channaveeraswamy, A. S., Vinod Kumar, D., RudraNaik, V. and Ashtaputre, S. A., 2019. Faster Chemical Methods to Determine Genetic Purity in Pigeon Pea (*Cajanuscajan* L. Mill sp.). Int.J.Curr.Microbiol.App.Sci., 8(3): 1378-1392.

McKee, 1973. Chemical and biochemical techniques for varietal identification. Seed Sci. Technol., 1: 181-199.

Meshram, L. D. and Rahangdale, S. L., 1988, Studies on phenol test in rice. Ann. Pl. Physiol., 2 (1):107-109.

Paulsen, M. R., Wigger, W. D., Litchfield, J. B. and Sinclair, J. B., 1989.Computer image analysis for detection of maize and soybean kernel quality factors. J. Agric. Eng. Res., 43: 93-101.

Ponnuswamy, A. S., Bhasakaran, M. and Sashri, G., 2003. Variety characterization in cotton by physical, chemical and biochemical methods. Training Manual on Varietal Characterization by Image Analysis and Electrophoresis.106-120.

Raju, P., Patil, S. B., Vasudevan, S. N., Ibrahim, Md. and Mathad, R. C., 2017.Characterization of parents and F_1 seeds of rice hybrid KRH-4 using different chemical tests and seed image analyzer. Agric. Update., 12 (TECHSEAR-5), 1261-1265.

Rosta, K., 1975. Variety determination in rice. Seed Science and Technology., 3: 161 - 168.

Saharan, V., 1991. Varietal identification in rice. M. Sc. (Agri.) Thesis, Haryana Agric. Univ., Hissar (India).

Sainis, J. K., Shouche, S. P. and Bhagat, S. G., 2009. Image analysis of wheat grains

developed in different environment and its implications for identification. J. Agric. Sci., 144(3): 221-227.

SambasivaRao, P., Murali Mohan Reddy, B., Bharathi, M. and Bayyapu Reddy, K., 2002, Varietal identification in groundnut (*Arachishypogaea* L.) by chemical tests and electrophoresis of total soluble seed proteins. Seed Tech. News., 32(1): 93.

Shahriar, M. H., Robin, A. H. K., Begum, S. N and Hoque, A., 2014. Diversity analysis of some selected rice genotypes through SSR based molecular markers. J. Bangladesh Agril. Univ., 12(2): 307–311.

Suchowilska, E. and Wiwari, M., 2006.Multivariate analysis of image descriptors of common wheat (*Triticumaestivum*) and spelt (*Triticumspelta*) grain infected by *Fusariumculmorum*. Int. Agrophysics. 20: 345-351.

Takahasi, N. and Hamaz, A. H. A., 1983.Diffrentiation of ecotypes in *Oryzasativa* L. I: Re-examination for colour reaction with phenol. Japan J. Breed., 33(3):243-250.

Tiwari, J. K., Rastogi, N. K., Chandrakar, P. K., Sarawgi, A. K. and Verulkar, S. B., 2013.Identification of rice varieties through chemical tests. Seed Res., 41(1):83-90.

Utku, H., Koksel, H. and Ozakara, R., 1998. Classification of barleys based on malting quality by image analysis. J. Inst. Brew., 104: 351-354.

Vanagamudi, K., Palaniswamy, V. and Natesan, 1988. Variety identification in rice: Examination of hulled grain. Seed Sci. Technol., 16 (2): 465-470.

Vanderburg, N. J. and Vanzwol, R. A., 1991. Rapid identification techniques used in laboratories of the International Seed Testing Association: a survey. Seed Sci Technol., 19: 687-700.

Vijayalakshmi, B. and Vijay, D., 2009. Development of Seed Keys for Varietal Identification in Rice (*Oryzasativa*) using Chemical Tests.Seed Research. 37 (1&2): 56-61.

Walls, W. E., 1965.A standardized phenol method for testing wheat seed for varietal purity.

Wang, J. and Shen, Z., 1992. Study on the classification of American rice varieties. Crop Gen. Resour., 2:4-6.

Zayas, I. Y., Martin, C. R., Steele, J. L. and Katsevich, A., 1996. Wheat classification using image analysis and crush force parameters. Ameri.Soci. Agric. Eng., 39 (6): 2199-2204.

Zayas, I. Y., Pomeranz, Y. and Lai, F. S., 1989.Discrimination of wheat and non wheat components in grain sample by using image analysis. Cereal Chem., 66 (3): 233-237.

How to cite this article:

Sudeep Kumar, E., S. N. Vasudevan, N. M. Shakuntala, S. R. Doddagoudar, B. G. MasthanReddy and Mahantashivayogayya, K. 2021. Characterization of Rice Genotypes Based on Physical, Chemical and Molecular Methods. *Int.J.Curr.Microbiol.App.Sci.* 10(01): 1480-1492. doi: https://doi.org/10.20546/ijcmas.2021.1001.176