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Characterization and Identification of Rice Germplasm Accessions Using Chemical Tests

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ABSTRACT: Variety characterization and identification has become invariably significant for purity maintenance during seed production as well as for the varietal protection under plant variety protection. In the present study, utility of chemical tests for the purpose was evaluated in rice using 155 germplasm accessions. Based on phenol colour reactions, 155 germplasm accessions could be grouped into dark brown, brown, light brown, black and no colour reaction categories comprising of 34, 23, 33, 17 and 48 in each germplasm accession, respectively. Further, germplasm accessions under each category were subjected to modified phenol test that helped in further to differentiate or identification of the rice germplasm accessions. Similarly, sodium hydroxide test and potassium hydroxide test have grouped the germplasm accessions into 2 and 4 colour categories, respectively.

Key words: Germplasm, Modified phenol test, Phenol test, Potassium hydroxide test, Sodium hydroxide test.

INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food in many parts of the world, including many developing countries in Asia, Africa, and Latin America. Ever growing population and decreasing agricultural resources may lead to food scarcity in near future. Hence, to maintain the sustainable rice production, persistent emphasis has been given for rice crop improvement since ages, which has resulted in release of large number of varieties with improved yield or tolerance to biotic and abiotic stresses.

Thus, the seed market currently offers a wider choice for the farmer for selecting a suitable rice variety depending on agro-climatic conditions of this region, commercial value of the variety, agronomic practices and cropping pattern to be followed with the particular variety. So in order to facilitate the farmer in selecting the variety of his choice, characterization and identification of a variety with suitable characters is important. Besides, variety characterization and identification meets multiple requirements of various stake holders such as for breeder to assess the diversity of breeding stocks. Similarly, for testing authorities, it facilitates determination of variety's uniqueness prior to their registration under PVP Act, whereas for certifying authorities, it is required for controlling the subsequent multiplication and marketing of seed lots. Most importantly, for farmers, selection of suitable variety for its commercial cultivation is inevitable and characterization is a pre-requisite for assisting in the decision-making process [1]. Conventionally, characterization and identification of a variety is done based on morphological characters, wherein plants of a variety were grown and observations on morphological characters were taken to establish the identity of the variety through characterization. However, though the morphological characters are the universally undisputed markers for varietal characterization and identification, at times these

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exhibit limitations, like the small variability observed between varieties, which result from the narrow genetic base of the germplasm used by the breeders, the environmental effect on the expression of the characters and the time required for obtaining results. Therefore, there is a need to screen for additional descriptors with significant discriminating power and reproducibility, that are not influenced by the environment [2]. Seed's colour reaction to different chemicals is one such alternative. When seeds are exposed to certain chemicals, they develop a characteristic colour depending on its chemical or metabolite constitution. This color is a variety specific trait and the application of this concept for the purpose of varietal characterization and identification has been well established in different crops. For instance, modified phenol test in wheat [3], KOH test in rice [4], $FeSO_4$ test in foxtail millet [5] pearlmillet [6] and sorghum [7] etc. These chemical tests are simple to perform, does not require any specialized technical expertise and when used along with other simple characters like seed and seedling characters, their varietal discrimination and identification power intensifies significantly [8]. Realizing this, glume phenol reaction (phenol test) has been included as one of the character for DUS testing in rice under DUS test guidelines of rice by both UPOV and PPVFRA [9-10].In rice, utilization of simple laboratory techniques like KOH test has been well established [4,11-12]. However, number of varieties used in such published work is limited and increase in number of released varieties over the time prompts for assessment of effectiveness of chemical tests for varietal characterization and identification. Hence, the present work has been undertaken with the objective of evaluating the effectiveness of the chemical tests in characterization and identification of rice varieties by taking 155 rice germplasm accessions.

MATERIAL AND METHODS

Phenol, modified phenol, potassium hydroxide and sodium hydroxide tests have been carried out at ICAR-Indian Institute of Seed Science, Mau, Uttar Pradesh using a set of 155 germplasm lines of rice. These germplasm accessions representing diverse genetic material, including land races, improved varieties from northern and southern India, *japonica* lines covering short, medium, long duration groups, irrigated and rainfed ecotypes, basmati, non basmati grain types and some resistant varieties to biotic and abiotic stresses (Table 1) were subjected to chemical test analyses.

Phenol Test

A total of two hundred (50 x 4) seeds were pre-soaked in distilled water for 24 h at 25 ± 1°C. Thereafter, the pre-soaked seeds were transferred on to two layers of Whatman No.1 filter paper saturated with 4% (w/v) phenol solution. The Petri dishes were covered and incubated at 25 ± 1°C and the change in seed glume along with aleurone layer colour was observed after 24 h [13-14].

Modified Phenol Test-A ($CuSO_4$)

Modified phenol test was conducted as per the phenol test except that seeds were soaked in 0.5% (w/v) $CuSO_4$ solution for 24 h instead of distilled water. Colour reaction of glume along with aleurone layer was observed after 48 h of incubation [13].

Sodium Hydroxide Test (NaOH)

Fifty seeds (50 x 4) of each germplasm accession (155) were pre-soaked in 5% (w/v) sodium hydroxide solution and kept at room temperature for 1 h. The change in colour of glume along with aleurone layer was observed based on the reaction of the seed with the test [15].

Potassium Hydroxide Test (KOH)

For the potassium hydroxide test (KOH), fifty seeds (50 x 4) of each germplasm accession (155) were pre-soaked in 5% (w/v) potassium hydroxide solution and kept at room temperature for 1 h. The change in colour of glume along with aleurone layer was observed based on the reaction of the seed with the test [16].

Table 1. List of Rice germplasm lines and its accession numbers

GP no.	Acc. no.	GP no.	Acc. no.	GP no.	Acc. no.	GP no.	Acc. no.
1	1001	40	1395	79	2734	118	3331
2	1003	41	1396	80	2842	119	3333
3	1005	42	1397	81	2857	120	3335
4	1007	43	1402	82	2875	121	3336
5	1012	44	1470	83	2885	122	3339
6	1013	45	1819	84	2889	123	3340
7	1015	46	1829	85	2894	124	3346
8	1016	47	1847	86	2919	125	3349
9	1018	48	1862	87	2922	126	3364
10	1024	49	1902	88	2924	127	3379
11	1025	50	1947	89	2925	128	3427
12	1031	51	1983	90	2927	129	3498
13	1034	52	2017	91	2942	130	3500
14	1039	53	2023	92	2943	131	3630
15	1046	54	2060	93	2973	132	3644
16	1059	55	2070	94	3060	133	3655
17	1161	56	2135	95	3073	134	3675
18	1167	57	2156	96	3075	135	3685
19	1183	58	2256	97	3082	136	3758
20	1193	59	2279	98	3102	137	3761
21	1201	60	2329	99	3112	138	3810
22	1202	61	2332	100	3118	139	3815
23	1204	62	2365	101	3120	140	3819
24	1206	63	2367	102	3122	141	3846
25	1213	64	2378	103	3126	142	3855
26	1221	65	2434	104	3127	143	3869
27	1224	66	2467	105	3128	144	3877
28	1226	67	2514	106	3129	145	4003
29	1228	68	2523	107	3138	146	NDR-359
30	1238	69	2541	108	3171	147	Sarjoo-52
31	1243	70	2545	109	3173	148	HUR105
32	1245	71	2575	110	3174	149	Kala namak
33	1259	72	2611	111	3264	150	MTU-7029
34	1264	73	2681	112	3287	151	PR(Punjab rice)-118
35	1265	74	2693	113	3295	152	BPT-5204
36	1356	75	2710	114	3296	153	PR-113
37	1381	76	2717	115	3319	154	PS(pusa sugandh)-3
38	1387	77	2723	116	3321	155	PS-5
39	1393	78	2729	117	3326	-	-

GP no.: Germplasms number; Acc. no.: Accession number

RESULTS AND DISCUSSION

Phenol Test

Conventionally, varietal identification involved visual inspection of morphological characters [Grow Out Test (GOT)] of plants with the aid of reference manual and systematic descriptors of national set of varieties [17-18]. Unlike GOT, the rationale in chemical tests relies on the differences of seed coat/pericarp composition that might be different due to genetic inheritability. In addition, environmental factors do play a key role in development of particular colour. Phenol test is one of the chemical tests, which are considered as a primary descriptor in identification of germplasm/variety owing to its high heritability and stability of the phenol colour reaction [11]. From the study, the 155 germplasm accessions showed different colour reaction with the phenol test (Table 2). Based on phenol colour reaction different rice germplasm accessions were distinguished into five distinct groups. Among 155 germplasm accessions, 33 showed light brown colour, 23 developed brown colour, 34 resulted with dark brown colour and 17 germplasm accessions showed black colour. Furthermore, some 48 germplasm lines showed no reaction to phenol test.

From the results, it is clearly observed that out of 155 germplasm accessions, 107 germplasm accessions showed different colour with phenol test. In a living tissue, phenol oxidation accomplishes by two reactions. In the first reaction, the aromatic ring of phenol can be hydroxylated to form catechols or quinols, respectively. In the second reaction, the quinols or catechols undergo oxidation to form quinones [19-20]. The germplasm accessions (107), which showed colour, are able to oxidize the phenol by tyrosinase enzyme, located at seed coat [17]. The extent of colour intensity among germplasm accessions were varied that might be due to differences in enzyme activity, temperature, light, aeration and genetic background, respectively [21-22]. On the other hand, the remaining germplasm accessions (48) showed no colour with phenol test. Takahashi and Hamza (1983) illustrated that the germplasm accessions were unable to hydroxylate the aromatic ring of phenol due to either shortage of electron donor or hydroxylating enzyme [23]. Phenol test could be a primary descriptor for able distinguishing nature of several varieties/germplasm accessions such as sarjoo-52, HUR 105, kala namak, MTU-7029, PR (Punjab rice)-118, BPT-5204, PR-113, PS-3 and PS-5 (Table 2). However, NDR-359 showed no reation to phenol test. Hence, it may be reasoned that phenol test could be used initially to group the germplasm accessions and upon testing with certain other tests could be more informative. Several reports corroborate the similar results with the study under investigation [11, 19,24-25].

Table 2. Varietal identification of 155 rice germplasms based on Phenol test

	No reaction(NR)	Light brown(LB)	Brown(B)	Dark black(DB)	Black(BL)
Total no. of germplasm lines of rice	**GP-**2, 4, 16, 17, 18, 26, 27, 33, 34, 35, 37, 39, 41, 42, 43, 44, 48, 49, 51, 60, 61, 62, 65, 70, 71, 74, 76, 77, 78, 82, 84, 87, 92, 93, 94, 95, 99, 103, 106, 107, 109, 119, 121, 128, 131, 132, 144 and 146	**GP-**1, 3, 5, 6, 15, 20, 24, 25, 28, 30, 31, 32, 40, 54, 55, 56, 58, 59, 66, 69, 75, 79, 111, 130, 136, 139, 140, 143, 147, 151, 153, 154 and 155	**GP** 13, 19, 47, 53, 57, 63, 68, 73, 81, 97, 100, 102, 104, 114, 117, 122, 123, 124, 125, 126, 134, 138 and 152	**GP-**4, 7, 9, 10, 12, 21, 22, 29, 36, 46, 50, 52, 67, 72, 83, 85, 86, 88, 90, 91, 96, 98, 101, 108, 112, 113, 116, 127, 129, 137, 141, 142, 145 and 148	**GP-**8, 11, 23, 38, 45, 64, 80, 89, 105, 110, 115, 188, 120, 133, 135, 149 and 150
	NR: 48	**LB: 33**	**B: 23**	**DB: 34**	**BL: 17**

GP-germplasm, No reaction (NR), Light brown (LB), Brown (B), Dark black (DB), Black (BL)

Table 3. Varietal identification of 155 rice germplasm accessions based on modified Phenol test

Phenol test	No reaction(NR)	Light brown(LB)	Brown(B)	Dark black(DB)	Black(BL)
A total of 48 germplasm lines No reaction (NR) with phenol test are subjected	GP-2, 14, 16, 17, 18, 26, 27, 33, 34, 37, 41, 42, 43, 48, 49, 51, 60, 61, 62, 65, 70, 71, 74, 76, 77, 78, 82, 84, 87, 94, 95, 99, 103, 106, 107,	GP-33, 92, 93,	None	GP-35	GP-44
	41	05	-	01	01
A total of 33 germplasm lines Light brown (LB) with phenol test are subjected to	None	GP-1, 6, 32, 54, 55, 59, 66, 69, 75,	GP-15, 20, 24, 25, 28, 30, 40, 58, 111, 136, 140, 143, 147,	GP-3, 5, 31,	None
Total	-	11	16	06	-
A total of 23 germplasm lines Brown (B) with	None	None	GP-63, 68, 97, 100, 102, 104,	GP-13, 19, 47, 53, 57, 73, 81, 122, 123, 124,	None
Total	-	-	09	13	-
A total of 34 germplasm lines Dark black (DB) with phenol test are subjected to modified	GP-21 and 36	GP-46 and 112	None	GP-4, 85, 98, 101, 129, 141, 142, 145 and	GP-7, 9, 10, 12, 22, 29, 50, 52, 67, 72, 83, 86, 88, 90, 91, 96, 108, 113, 116,
Total	02	02	-	09	21
A total of 17 germplasm lines black (BL) with phenol test are subjec-ted to modi-fied	GP-45	None	GP-105	None	GP-8, 11, 23, 38, 64, 80, 89, 110, 115, 118, 120, 133, 135,
Total	01	-	01	-	15

Modified Phenol Test

Modified phenol test is one more promising test that augments the colour of the phenol reaction [26]. The germplasm accessions, which showed no colour (48) with phenol test were treated with modified phenol test. Intriguingly, out of 48 germplasm accessions, 5 germplasm accessions showed light brown colour, 01 germplasm resulted in dark brown and 01 additional germplasm gave black colour. The remaining 41 germplasm accessions showed no colour with the modified phenol test (Table 3). The colour development in modified phenol test is due to Cu^{++} ions which may act as co-factor for the hydroxylating enzyme [13, 4, 27-28]. Further, the germplasm accessions which showed positive result with phenol test were subjected to modified phenol test to differentiate further. A majority of germplasm accessions responded positively to the modified phenol test with $CuSO_4$ (Table 3). For instance, 33 germplasm accessions showing light brown colour with phenol test were treated with modified phenol test; where 11 showed light brown, 16 germplasm accessions demonstrated brown colour and 6 germplasm accessions resulted in dark brown colour. Similarly, 23 germplasm accessions that showed black colour were subjected to modified phenol test, of which, 9 germplasm accessions were black and 13 germplasm accessions showed dark brown colour. Furthermore, 34 germplasm accessions demonstrated dark brown colour after exposure to modified phenol test; where 2 germplasm accessions showed light brown, 9 germplasm accessions were dark brown, 21 germplasm accessions developed black coloration and the rest 2 germplasm accessions showed no reaction. From the studies, the phenol test alone may not possess good discriminating power, but have several advantages like cost-effectiveness, ease of performance, quite helpful in identifying particularly in a large number of seed lots. However, phenol test coupled with modified phenol test could be a viable option to group and identify the varieties, which are simple, cheap and quick method to distinguish the rice germplasm accessions.

Sodium hydroxide and Potassium hydroxide Tests

Sodium hydroxide and potassium hydroxide tests are quite helpful to differentiate white seeded varieties, in cases, when red seeded varieties lose seed coat colour owing to unfavourable climatic conditions. In the present study, the 155 germplasm lines of rice were grouped into two distinct classes based on their colour reaction with sodium hydroxide test, where 144 germplasm accessions showed light yellow and 11 germplasm accessions resulted in wine red colour (Table 4).

Similarly, when the germplasm accessions were treated with potassium hydroxide, 82 have showed light yellow, 25 resulted in dark yellow,

Table 4. Varietal identification of 155 rice germplasm accessions based on Sodium Hydroxide test (NaOH)

	Light Yellow	Wine Red
Total number of germplasm lines of rice	**GP-** 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 26, 27, 28, 29, 30, 31, 32, 34, 36, 37, 39, 40, 41, 42, 43, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 65, 66, 67, 69, 70, 71, 72, 73, 75, 76, 77, 78, 79, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154 and 155	**GP-**12, 25, 33, 35, 38, 44, 45, 64, 68 74 and 80
	LY: 144	**WR: 11**

GP-germplasm, Light Yellow (LY), Wine Red (WR)

Table 5. Varietal identification of 155 rice germplasm accessions based on Potassium hydroxide test (KOH)

	Light Yellow	Dark Yellow	Light Wine Red	Dark Wine Red
Total no. of germplasm lines of rice	**GP**- 2, 3, 4, 8, 9, 10, 11, 13, 14, 16, 17, 19, 20, 21, 22, 24, 26, 28, 29, 30, 32, 34, 36, 39, 40, 41, 42, 44, 48, 50, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 69, 70, 72, 75, 76, 80, 81, 82, 84, 85, 86, 87, 88, 91, 92, 95, 96, 97, 99, 100, 101, 102, 103, 104, 105, 106, 109, 113, 114, 116, 121, 123, 125, 126, 127, 130, 139, 142, 144, 146, 147, 151, 152 and 153	**GP**-33, 39, 52, 66, 73, 79, 83, 111, 117, 118, 119, 122, 128, 135, 136, 137, 138, 140, 141, 143, 145, 148, 150, 154 and 155	**GP**-5, 6, 7, 12, 15, 18, 23, 25, 27, 31, 35, 37, 43, 46, 65, 67, 68, 71, 74, 77, 89, 93, 98, 107, 108, 110, 115, 129, 131, 132, 134 and 149	**GP**-1, 45, 47, 49, 51, 64, 78, 90, 94, 112, 120, 124 and 133
	LY: 85	**DY: 25**	**LWR:32**	**DWR: 13**

GP-germplasm, Light Yellow (LY), Dark Yellow (DY), Light Wine Red (LWR), Dark Wine Red (DWR)

32 revealed light wine red and 13 recorded dark wine red colour, respectively (Table 5). The reasons for variation in colour might be due to inherent chemical difference, stability of genetic characters and secondary metabolites present in the seeds [17]. Similar results were reported by Nethra *et al.* [12]; Dileepkumar *et al.* [24]; Vijayalakshmi and Vijay [29].

Hence, based on the colour reaction with phenol and modified phenol tests the germplasm accessions can be categorized into various groups and the standard phenol test with $CuSO_4$ (modified phenol test) was found to be better in distinguishing germplasm lines of rice.

Phenol test, which is an index of polyphenol oxidase activity, has been reported to be associated with intra-varietal diversity and have been used in ascertaining varietal purity. This reaction caused melanin formation by oxidizing phenol via orthoquinones and hydroxyquinones. This reaction is controlled by single gene (monogenically), which is localized in seed coat. Therefore, it was considered as important primary descriptor for grouping and identification of germplasm lines of rice. The findings were in accordance with the report by Rao *et al.* [30].

CONCLUSIONS

Based on the response of 155 germplasm lines of rice to chemical tests, a comprehensive key has been developed for rapid identification of rice germplasm accessions. In the present study, chemical tests namely phenol, modified phenol, potassium hydroxide and sodium hydroxide tests were carried out to develop additional descriptors to differentiate the 155 rice germplasm accessions. From the studies, phenol test is able to group the germplasm accessions and further enhancement was done with modified phenol test. Sodium hydroxide could be employed to identify the red seeded varieties. Importantly, it has been observed that single chemical test may not be enough to identify the germplasm accessions and several tests need to be employed in a complementary manner. In addition, these chemical tests were highly stable, rapid, cost effective and are least influenced by the environment [31-32].

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