VARIETAL IDENTIFICATION AND CHARACTERIZATION BASED ON CHEMICAL TESTS IN DIFFERENT INDICA RICE VARIETIES OF NORTH WESTERN HIMALAYAS

Abstract

Variety identification has great significance from seed production, breeding as well as intellectual property rights point of view to ensure quality seed. The decrease in the morphological variation among the major rice varieties due to narrow genetic base makes it necessary for the development of quick and reliable tests for varietal identification particularly for those working in seed certification and quality maintenance. The study was conducted during 2019-20 at the laboratories of the Crop Improvement Division, ICAR-VPKAS, Almora.Two hundred Indica rice varieties/genotypes were identified on the basis of seed color (phenol, modified phenol, NaOH and KOH~~) to~~ chemical tests).Phenol test grouped variety into three distinct groups viz., dark brown (15 varieties), brown (59 varieties) and light brown (95 varieties). With the help of modified phenol test these varieties were further sub grouped. NaOH test grouped varieties into two distinct groups i.e., light yellow (177 varieties) and wine red (23 varieties). These tests clearly differentiated the varieties of one group to that of another groups on the basis of seed coat color. However, KOH test was not reliable for the differentiation of these varieties because all these varieties showed light yellow color after treating with KOH solution. Though no individual chemical test was able to distinguish all the varieties, different chemical tests in conjunction were useful in identification of varieties. Thus these simple, reliable and quick tests can be used for varietal identification in rice crop.

Keywords: Rice varieties, Chemical tests, phenotype, Seed color

Introduction

Rice (*Oryza sativa* L. 2n = 24) belongs to the family *Graminae* and sub family *Oryzoidea*. Rice has a renowned relationship with the humans since ages. Presently, more than half of the world's population of 7.8 billion (Worldmeters 2020; UN 2020) depends on rice as a staple food. Asia can be considered as 'Rice Basket' of the world, as more than 90 per cent of the rice is produced and consumed in Asia, a region with high population density. Besides dominating as an indispensable food component in Asia, rice is rapidly emerging as the chief food in Latin America and Africa.

In India,Rice is an important food crop and highest numbers of varieties were released in rice among different crop specific improvement programmes, to cater the needs of the farmers and consumers. But with time only few varieties got stabilized and occupied major rice growing

areas of the country. Characterization of cultivars, establishment of varietal identity and genetic purity of the seed lots are crucial for varietal improvement, varietal protection and seed production. Due to narrow genetic base and reduced range of variation among varieties chosen by the farming community coupled with the interest of consumers towards specific grain types (Medium and long slender) of rice varieties, it is often becomes difficult to identify the varieties just by using morphological characteristics, especially for those involved in seed certification and quality maintenance. Thus varietal identification becomes an essential issue to maintain the genetic purity and identity of each variety. A rapid and reliable technique to verify the identity and to assess the purity of seed lots is important in seed quality assurance programme to meet out the minimum seed certification standards of seed quality prescribed for the certified class.

Hence, in order to establish and maintain a high market reputation, all major seed companies, both in private and public sectors, take necessary care to ensure highest quality standards. Verification of the seed purity is essential before marketing of commercial seed.

The aspect of Distinctness, Uniformity and Stability (DUS) is fundamental for characterization of varieties. Accurate identification of varieties is not only a pre requisite for DUS testing, but is critical for the production of quality seed also. The most common method of varietal identification is grow out test (Vishwanath *et al.,* 2013 and Arus*et al.,* 1982), which is based on the morphological markers at various growth stages of plant, however it has many disadvantages such as interaction with environment, high cost and lengthy time required, low polymorphism in closely related varieties which were bred by using elite lines, to name but a few. The time duration of one complete season for identification of varieties using standard grow-out test is one of the major constraints in its implementation, particularly in the major rice growing regions of North Western Himalayas. Moreover high number of new cultivars and their mutual similarity do not allow their differentiation based on phenotype. It is less suitable when the results are required rapidly and is influenced by environment because of its mutagenic and continuous expression. Recent discoveries in the area of biochemistry and molecular biology have enabled seed scientist to utilize new techniques for cultivar identification to augment existing traditional methods. Proteins, isozymes and DNA markers are independent of environmental factors, these markers have been used to characterize (Tanksley *et al.* 1992; Lucchese *et al*., 1999, Vishwanath *et al*., 2010). However, these require high investment cost, and sophisticated lab facility and skilled personnel. Therefore it is necessary to find simple and cost effective techniques for characterization and identification of rice genotypes. Reaction of seeds or seedlings to different chemicals is based on seed composition; this property has been used by several researchers to characterize many crop cultivars (Papp *et al*., 1997, Vanangamudi *et al*., 1998, McDonald Jr., 1985, Punia *et al*., 2002, Patil *et al*., 2006; Nethra *et al*., 2007). These chemical tests are very quick, easy to do, reproducible and can be conducted throughout the year under controlled conditions. Hence several simple and rapid chemical tests were developed to unlock the ambiguity in varietal identification in rice. Some of the popular chemical tests used in Indian mustard for varietal characterization are phenol test, modified phenol test (CuSO4 and

Na2CO3), sodium hydroxide (NaOH) test, peroxidase test, potassium hydroxide (KOH) test and 2, 4-D auxin test. The chemical tests reveal differences of color among the seeds. In these chemical tests, the chemical agents react with the seed and help in varietal identification. They require virtually no technical expertise or training and can be completed in a relatively short time. The present investigation was undertaken to develop seed keys for the identification of different rice genotypes using various simple and rapid chemical tests in conjunction.

MATERIALS AND METHODS

The freshly harvested pure seeds of two hundred rice genotypes/varieties grown at Hawalbagh Experimental Farm, ICAR-VPKAS, Almora were used for the present study (Table 1). The experiment was conducted at the Crop Physiology Laboratory of Institute during the period of 2019-20. Four chemical tests viz. Phenol, modified phenol, Potassium hydroxide and sodium hydroxide tests were conducted on the rice grains. The list of varieties/genotypes is given below (Table 1):

Phenol test

The Standardized phenol test for varietal purity testing as suggested by Walls (1965) was followed. Four replications of 100 seeds were soaked in distilled water for 24 hours. 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 color was observed after 24 h [13-14]. After that, the seeds were examined and grouped into different color classes as no color change, light brown, brown, dark brown and black.

Modified Phenol Test-A ($CuSO_4$)

As described by Banerjee and Chandra (1977), the procedure similar to the standard phenol test except that the seeds were soaked in a solution of 0.5% (w/v) $CuSO_4$ solution for 24 h instead of distilled water. Color reaction of glume along with aleurone layer was observed after 48 h of incubation [13]. The seeds were examined and grouped into five distinct groups namely, no color change, light brown, brown, dark brown and black.

Sodium Hydroxide Test (NaOH)

Fifty seeds (50 x 4) of each genotype/variety (200) were pre-soaked in 5% (w/v) sodium hydroxide solution and kept at room temperature for 1 h. The change in color of glume along with aleurone layer was observed based on the reaction of the seed with the test (Chakrabaurty and Agarwal, 1989).

Potassium Hydroxide (KOH) test

Fifty seeds in four replications (50 x 4) of each genotype/variety (200) were pre-soaked in 5% (w/v) potassium hydroxide solution and kept at room temperature for 1 h. The change in color of glume along with aleurone layer was observed based on the reaction of the seed with the test [15].

Table1. List of two hundred rice germplasm accessions evaluated in this study

GP No.	Variety	GP No.	Variety	GP No.	Variety	GP No.	Variety
1.	VL 32637	51.	VL 32525	101.	VL 40657	151.	VL 20926
2.	VL 32650	52.	VL 32558	102.	Pusa Bas -1	152.	VL 20929
3.	VL 32651	53.	VL 32560	103.	Tarawari	153.	VL Dhan 206
4.	VLD 86	54.	VL 32573	104.	Pusa 1121	154.	VL Dhan 207
5.	VL 32652	55.	VL 32574	105.	Pusa Sungdh-2	155.	VL Dhan 208
6.	VL 32678	56.	VL 32577	106.	VL 40674	156.	VL Dhan 209
7.	VL 32699	57.	VL 32580	107.	VL 40678	157.	VL Dhan 221
8.	VL 32603	58.	VL 32585	108.	VL 40686	158.	Vivek Dhan 154
9.	VL 32604	59.	VL 32594	109.	VL 40687	159.	VL Dhan 155
10.	VL 32605	60.	VL 32596	110.	VL 40688	160.	VL Dhan 156
11.	VL 32606	61.	VL 32735	111.	VL 40690	161.	VL Dhan 157
12.	VL 32608	62.	VL 32740	112.	VL 40696	162.	VL Dhan 158
13.	VL 32622	63.	VL 32742	113	VL 40698	163.	VL Dhan 81
14.	VL 32623	64.	VL 32745	114.	VL 40703	164.	Vivek Dhan 82
15.	VLD 68	65.	VL 32751	115.	VL 40705	165.	VL Dhan 85
16.	VL 32635	66.	VL 32754	116.	VL 40708	166.	VL Dhan 86
17.	VL 32636	67.	VL 32759	117.	VL 40713	167.	VL Dhan 87
18.	VL 32654	68.	VL 32760	118.	Pusa Sug-2	168.	VL Dhan 61
19.	VL 32665	69.	VL 32763	119.	Tarawari Bas	169.	Vivek Dhan 62
20.	VL 32736	70.	VL 32765	120.	Pusa Bas-1	170.	VL Dhan 65
21.	VL 32737	71.	VL 32766	121.	Bas 1121	171.	VL Dhan 68
22.	VL 32739	72.	VL 32767	122.	VL 40660	172.	VL Dhan 8
23.	VL 32741	73.	VL 32768	123.	VL 40662	173.	VL dhan 16
24.	VL 32743	74.	VL 32777	124.	VL 40663	174.	VLK -39
25.	VL 32744	75.	VL 32778	125.	VL 40665	175.	VL 11364
26.	VL 32753	76.	VL 32779	126.	VL 40670	176.	VL 11574
27.	VL 32756	77.	VL 32780	127.	VL 40671	177.	VL 20073
28.	VL 32769	78.	VL 32782	128.	VL 40672	178.	VL 20083
29.	VL 32802	79.	VL 32793	129.	VL 40673	179.	VL 32094
30.	VL 32829	80.	VL32 803	130.	VL 40675	180.	VL 32130
31.	VL 32835	81.	VL 32828	131.	VL 40680	181.	VL 32224
32.	VL 32836	82.	VL 32831	132.	VL 40681	182.	VL 32237
33.	VL 32838	83.	VL 32832	133.	VL 40689	183.	VL 20725
34.	VL 32848	84.	VL 32840	134.	VL 20607	184.	VL 20728
35.	VL 32850	85.	VL 32842	135.	VL 20608	185.	VL 20729
36.	VL 32863	86.	VL 32847	136.	VL 20615	186.	VL20808
37.	VL 32865	87.	VL 32848	137.	VL 20616	187.	VL 20811
38.	VL 32867	88.	VL 32858	138.	VL 20618	188.	VL 20816
39.	VL 32868	89.	VL 32864	139.	VL 20620	189.	VL 20818
40.	VL 32473	90.	VL 32866	140.	VL 20703	190.	VL 20254
41.	VL 32474	91.	VL 32869	141.	VL 20705	191.	VL 20611

42.	VL 32475	92.	VL 40589	142.	VLD 157	192.	VL 20612
43.	VL 32510	93.	VL 40590	143.	Vivek Dh 154	193.	VL 20684
44.	VL 32511	94.	VL 40593	144.	VL 20876	194.	VL 20687
45.	VL 32514	95.	VL 40594	145.	VL 20890	195.	VL 32292
46.	VL 32517	96.	VL 40606	146.	VL 20899	196.	VL 32308
47.	VL 20685	97.	VL 40611	147.	VL 20922	197.	VL 32462
48.	VL 32515	98.	VL 40627	148.	VL 20923	198.	VL 32465
49.	VL 32521	99.	VL 40648	149.	VL 20924	199.	VL 32471
50.	VL 32522	100.	VL 40650	150.	VL 20925	200.	VL 40530

(Source: Germplasm bank, ICAR-VPKAS)

RESULTS AND DISCUSSION

Developments of various techniques in order to identify the varieties in the laboratory are beset with many difficulties and a single technique may not be adequate correctly to identify a variety. Under such conditions a number of tests in combination can resolve themselves into a key for identification of varieties. Seeds constituents vary between the cultivars. The reaction of these constituents with chemicals resulted in different reaction. Various biochemical tests are being used to reveal chemical differences among the seeds or seedlings of different cultivars. They require virtually no technical expertise or training and can be completed in a relatively short time. The results of these tests are usually distinct, easily interpreted and helps in distinguishing or grouping of cultivars. Keeping this in view, a critical evaluation of various techniques has been employed for identification of rice varieties.

A. Phenol Test

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 color reaction [11]. From the study, the 200rice varieties/genotypes showed different color reaction with the phenol test (Table 2, Fig 1). Based on the color development of the decanted solution for the Phenol test (1%), different rice genotypes were distinguished into five distinct groups. Among 200 germplasm accessions, 95 showed light brown color, 59 resulted with brown color and 15 genotypes developed dark brown color and no germplasm accessions showed black color. Furthermore, some 31 germplasm lines showed no reaction to phenol test. From the results, it is clearly observed that out of 200 germplasm accessions, 169varieties showed different color with phenol test.

Phenol test showed great variation among varieties into light brown, brown and dark brown group (Table 2, Fig 1). This test is highly specific for varieties. Phenol reaction is monogenically controlled response, which is present in seed coat (Joshi and Banerjee, 1970). An enzyme polyphenol oxidase (PPO) is responsible for the oxidation of externally supplied phenol into quinones and their further polymerization yield melanin like pigments catalysed primarily by the enzyme tyrosinase in the seed coat and have resulted in development of brown coloration in seeds. The results are in conformity with findings of Singh *et al.* (2017), Tiwari *et al.* (2013).

Table 2. Varietal identification of 200 rice germplasm accessions based on Phenol test

	No reaction(NR)	**Light Brown(LB)**	**Brown(B)**	**Dark Brown(DB)**	**Black(BL)**
Total no. of germplasm lines of rice	GP-20,21,22,37,38, 61,89,107,108,109, 110,112,122, 123,132,133,134, 137,139,146,153 156,157,160,161 162,175,176,178 180,194	GP-3,4,5,6,9,11,12, 13,14,16,17,18,25 27,28,30,31,34,35 36,39,41,42,43,45 46,48,49,50,51,52 53,55,56,57,58,64 66,68,72,74,75,76 78,80,81,82,83,84, 85,86,88,90,97,103 104,105,106,111, 120,121,125,126, 127,128,129,130 142,143,144,154, 155,158,159,163 166,167,168,169, 170,171,172,177 179,181,182,183 184,185,186,193 195,196,198	GP-1,2,7,8,10,15 19,23,24,26,29,32 33,40,47,54,59,60 62,63,65,67,69,70 71,73,77,79,87,91 92,93,94,95,96, 102,115,116,117, 118,119,124,131, 135,136,140,141, 145,152,164,165 174,187,188,189 190,191,192,200	GP-44,98,99,100 101,113,114,138 147,148,149,150 151,173,199	GP-**NA**
	NR-31	**LB- 95**	**B-59**	**DB-15**	**BL-0**

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

B. Modified phenol test

The modified phenol test using $CuSO_4$ solution helped in further sub-division that augments the color of the standard phenol groups [4]. All the selected genotypes responded positively to the modified phenol test with CuSO4.

The genotypes, which showed no color (GP-37,38,122,123,157,178 and 180) with phenol test intriguingly showed light brown color when treated with modified phenol test. The presence of metallic ions Cu++ in modified test enhances the phenol color since it acts as a co-factor/catalyst for the hydroxylating enzyme [13, 4, 27-28] (Banerjee and Chandra, 1977).Further, the modified phenol test using $CuSO_4$ solution helped in further sub-division of standard phenol groups [4]. A majority of germplasm accessions responded positively to the modified phenol test with CuSO4 (Table 3, Fig 2). For instance, 95 germplasm accessions showing light brown color with phenol test were treated with modified phenol test; where 44 showed light brown, 24varieties demonstrated brown color, 24cultivars showed dark brown color, 5 cultivars resulted in black color and the rest 6 germplasm accessions showed no reaction..Furthermore, 59 germplasm accessions demonstrated brown colorin phenol test when exposed to modified phenol test; 3genotypes showed light brown, 15 were dark brown, 22 accessions developed black coloration. The rice variety GP No.173 (VL Dhan 16) which showed

dark brown when treated with Phenol test resulted in light brown color under modified phenol test. The remaining 30 genotypes showed no color with the modified phenol test (Table 3). 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. Hence, based on the color reaction with phenol and modified phenol tests the genotypes can be classified into different groups and the standard phenol test with CuSO4 found to be better in distinguishing cultivars. Similar observations were recorded by Gupta *et al*., (2007) in wheat and Anitalakshmi*et al*., (2014) in rice.

Table 3. Varietal identification of 200 rice germplasm accessions based on Modified Phenol test ($CuSO_4$)

	No reaction(NR)	**Light Brown(LB)**	**Brown(B)**	**Dark Brown(DB)**	**Black(BL)**
Total no. of germplasm lines of rice	GP-20,21,22 61,89,90,107 108,109,110 111,112,132 133,134,137 139,142,144 146,153,156 160,161,162 175,176,183 194,198 **NR-30**	GP-33,37,38,39,42 43,49,50,58,73,74,75 76,78,79,80,81,83,84 85,86,88,120,121 122,123,125,126,154 157,159,163,168 172,178,179,180 182,186,192,193 195,196,197 **LB- 44**	GP-7,18,19,26,27 28,36,40,41,44,46 48,52,53,54,56,60 62,64,65,66,71,72 77,82,87,135,136 143,145,155,158 165,166,167,169 170,171,173,177, 181,184,185,187 190,200 **B-46**	GP-1,2,3,4,5,6,8,9,11, 12,13,14,15,16,17,23 24,25,30,31,34,35,45 47,51,55,57,59,63,67 68,69,70,97,98,113,114 115,128,129,130,152 174 **DB-43**	GP-10,29,32,91,92,93 94,95,96,99,100,101 102,103,104,105,106,116 117,118,119,124,127, 131,138,140,141,147 148,149,150,151,164 188,189,191,199 **BL-37**

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

C. 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 color owing to unfavorable climatic conditions. In the present study, the 200 germplasm lines of rice were grouped into two distinct classes based on their color reaction with sodium hydroxide test, where 177 germplasm accessions showed light yellow and 23 germplasm accessions resulted in wine red color (Table 4, Fig 3). Likewise, all the two hundred germplasm accessions were grouped into four distinct classes based on their color reactions with KOH. These were namely Dark Wine red, Wine red, Light yellow, and Dark yellow .When the germplasm accessions were treated with potassium hydroxide, all the accessions shows Light yellow color (Table 5, Fig 4). Sodium hydroxide could be employed to identify the red seeded varieties. NaOH test could also resolve varieties from very strong groups of phenol and modified phenol tests and can be used as an alternative test in subdividing those groups. The reasons for variation in color might be due to inherent

chemical difference, stability of genetic characters and secondary metabolites present in the seeds (MASUTHI et al. 2015). Similar results were reported by Nethra *et al*.; Dileepkumar*et al*.; Vijayalakshmi and Vijay.

Table 4. Varietal identification of 200 rice germplasm accessions based on Sodium Hydroxide (NaOH) test

	Light Yellow	Wine red
Total number of germplasm lines of rice	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26, 27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,4 9,50,51,52,53,54,55,56,57,58,59,60,61,62,63,64,65,66,67,68,69,70,71, 72,73,74,75,76,77,78,79,80,81,82,83,84,85,86,87,88,89,90,91,92,93,9 4,95,96,97,98,99,100,101,102,103,104,105,106,107,108,109,110,111, 112,113,114,115,116,117,118,119,120,121,122,123,124,125,126,127, 128,129,130,131,132,133,134,135,136,139,140,141,142,145,153,157, 159,160,161,162,163,164,165,166,167,168,169,170,171,172,175,179, 181,182,183,185,186,187,189,190,191,192,193,194,195,196,197,198, 199,200. **LY-177**	137,138,143,144, 146,147,148,149 150,151,152,154 155,156,158,173 174,176,177,178 180,184,188 **WR-23**

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

Table 5. Varietal identification of 200 rice germplasm accessions based on Potassium Hydroxide (KOH) test

	Light Yellow	Dark Yellow	Light Wine Red	Dark Wine Red
Total no. of germplasm lines of rice	GP- 1 TO 200 **LY-200**	GP-0 **DY-0**	GP-0 **LWR-0**	GP-0 **DWR-0**

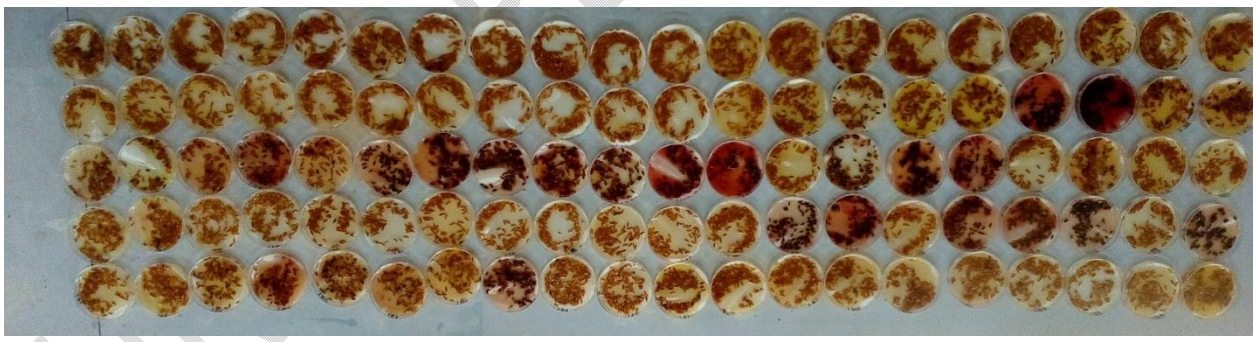
GP- germplasm, Light Yellow (LY), Dark yellow (DY), Light wine red (LWR), Dark Wine red (DWR)

Figures:

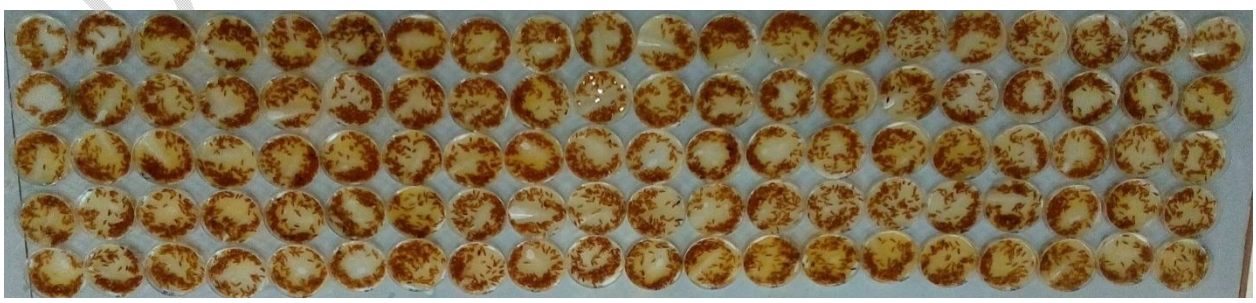
1. Phenol Test

2. Modified Phenol Test

3. NaOH TEST



4. KOH test



CONCLUSIONS

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 200 rice germplasm accessions. Based on the response of 200 germplasm lines of rice to chemical tests, a comprehensive key has been developed for rapid identification of rice germplasm accessions. In conclusion, it was observed that the statement, "individually chemical tests are of limited value, but when used in conjunction with each other can separate almost any number of rice varieties (Gupta *et al.* 1987) was proven in the present study where no single chemical test could distinguish even a single variety but when these tests were used in combination could identify all the two hundred varieties individually. Hence these simple, rapid and reliable tests are of immense value for the varietal identification purpose in rice crop. In addition, these chemical tests were highly stable, rapid, cost effective and are least influenced by the environment.

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