

Development of Seed Keys for Varietal Identification in Rice (*Oryza sativa*) using Chemical Tests

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ABSTRACT 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. Twenty three genotypes of rice were tested for five different chemical tests viz., Phenol, Modified Phenol, NaOH, FeSO₄ and KOH tests. Based on the colour reaction of different genotypes seed keys were developed for distinguishing the twenty three paddy varieties. All the five chemical tests grouped the varieties into two to four classes based on their reaction. Even though, not even a single test could identify any individual variety, but in conjunction the five chemical tests identified all the twenty three rice varieties individually. Thus these simple, reliable and quick tests can be used for varietal identification in rice crop.

Key words: Rice, chemical tests, seed keys, varietal identification.

Rice is an important food crop in India and highest numbers of varieties were released in rice, among crop species, 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. 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. 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 Andhra Pradesh where rice is grown in both *Kharif* and *Rabi* seasons. Hence several simple and rapid chemical tests were developed to unlock the ambiguity in varietal identification in rice.

Among different chemical tests developed the most popular one is phenol colour reaction which was used for determining the varietal identification in number of crops viz., wheat [1], rice [2], pearl millet [3], sorghum [4], foxtail millet [5] etc. Many researchers have used other chemical tests viz., modified phenol test [1], KOH test [2], FeSO₄ test [5] etc. along with phenol test for the development of seed keys. 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

Pure seeds of twenty three rice genotypes grown at A.P. Rice Research Institute, Maruteru were used for the present study. Five chemical tests viz. Phenol, modified phenol, Ferrous Sulphate, Potassium hydroxide and sodium hydroxide tests were conducted on the rice grains. Fifty seeds of each genotype were observed visually for seed colour with the aid of magnifying glass.

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Phenol test

The standard phenol test for varietal purity testing suggested by Walls [6] was followed. Four replicates of 50 seeds each were soaked in distilled water for 18 hours. 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 hours, the seeds were examined for staining and grouped in to very strong (++++), strong (+++), moderate (++) and negative. (-) based on the colour intensity.

Modified phenol test

The test suggested by Banerjee and Chandra [7] was followed. Four replications of 50 seeds each were soaked in 0.5 per cent solution of copper sulphate under ambient conditions for 18 hours and then placed over a filter paper moistened with 4 ml of 29 per cent phenol solution. The colour reaction observation was made in the similar manner as described for phenol test.

Ferrous sulphate test

As described by Gupta and Agrawal [8], fifty seeds each in four replications were soaked in 1.5 per cent FeSO_4 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 grouped as Dark Grey Streaks (DGSt), Brown Streaks (BSt) and Brown Spots (BSp).

Potassium hydroxide 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 (+Ve) and no staining (-ve) [9].

Sodium hydroxide 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 the

varieties were classified into three groups viz., Dark Yellow (DY), Light Yellow (LY) and no colour reaction (-) [10].

RESULTS AND DISCUSSION

The Phenol colour test, where phenol gets oxidised into dark colour melanin catalysed by tyrosinase enzyme, is a simple, quick and accurate test for grouping of rice varieties [11, 12]. Based on phenol colour reaction all the twenty three varieties used in the present study were grouped into four classes (Table 1). A similar type of classification was reported by number of workers in rice [9, 13, 2]. The maximum number of varieties (eleven) showed moderate colour reaction towards phenol test followed by no colour reaction and strong reaction (5 varieties each). The remaining two varieties viz., Varalu and Chenengi showed very strong reaction. In view of the high heritability and stability, phenol test was used as primary diagnostic character for distinguishing paddy varieties [8]. In the current investigation, two varieties, Tolakari and Chaitanya, with similar grain morphology were distinguished by phenol test. A close observation of the results indicate that even though this is a quick and stable test, it alone is not sufficient to identify all the varieties since it forms different groups based on their colour reaction. Therefore it is necessary to use various other chemical tests for distinguishing all the varieties.

The modified phenol test individually separates all the twenty three genotypes into four groups similar to phenol test. In the present investigation maximum varieties (eight) were grouped under very strong reaction class followed by strong, moderate and no colour reaction groups with five varieties each. The addition of Cu^+ ions in the modified test greatly enhances the colour reaction in a number of varieties as was found with the increase in number of varieties in the very strong group. Similar kind of results were reported by Jaiswal and Agrawal [13] in rice. The modified phenol test in combination with phenol test further subdivided within the groups formed by phenol test. The big group of varieties (11 No.) showing moderate reaction in phenol test was subdivided into four

Table 1. Rice varieties showing different colour reactions to chemical tests

S.No.	Name of the variety	Phenol test			Modified phenol test			FeSO ₄ test			KOH test		NaOH test				
		Very strong	Strong	Mode-rate	No color	Very strong	Strong	Mode-rate	No color	DGSt	BSt.	BSp	Deep wine red	No color	DY	LY	No color
1	Chaitnya				(-)				(-)		BSp		-ve				(-)
2	Maruteru Sannalu				(-)				(-)		BSt.		-ve				(-)
3	Vijetha		++						(-)		BSt.		-ve		LY		
4	Tholakari		++				++				BSp		-ve		LY		
5	Vajaram				(-)				(-)		BSt		-ve		LY		
6	Swarna		++			++++				DGSt			-ve		LY		
7	Deepthi				(-)				(-)		BSp		-ve		LY		
8	Krishanveni	+++				++++				DGSt			-ve	DY			
9	MTU 1004		++			+++					BSt		-ve		LY		
10	Anjali		++				++				BSp		-ve				(-)
11	Vikas	+++				++++				DGSt			-ve		LY		
12	Rajendra		++			++++					BSt.		-ve	DY			
13	ASD-7	+++				++++					BSp		+ve	DY			
14	PR-113		++			++++					BSt.		-ve				(-)
15	QPE-2		++			+++				DGSt			-ve		LY		
16	Rathuheenathi				(-)		+++			DGSt			+ve		LY		
17	Mudgo		++			+++					Bst.		+ve	DY			
18	Tadukan	+++				+++					BSt.		-ve		LY		
19	Varalu	++++				++++				DGSt			-ve		LY		
20	CO-31		++			+++					BSp		-ve	DY			
21	Pooja		++			+++					BSp		-ve	DY			(-)
22	Chenengi	++++				++++					BSp		+ve		LY		
23	Supreme	+++				++++				DGSt			-ve				(-)

Very strong: ++++; DGSt: Dark grey streaks; DY: Dark yellow; Strong: +++; BSt: Brown streaks; LY: Light yellow; Moderate: ++; BSp: Brown spots; No colour: +

classes viz., very strong (2 varieties), strong (3 varieties), moderate (5 varieties) and no colour (1 variety) by modified phenol test and similar is the case with other phenol groups.

Based on Ferrous sulphate test all the twenty three varieties were classified into three groups with seven varieties showing dark grey streaks and eight varieties each showing brown streaks and brown spots. Similar kind of results were obtained by Saharan [14] in rice where he classified 33 varieties into five groups. The ferrous sulphate test when used in combination with both phenol and modified phenol tests, could be able to distinguish eleven different varieties altogether (Fig. 1).

All the twenty three varieties were grouped into two distinct classes based on their colour reaction with KOH. Only four varieties developed deep wine red colour when treated with KOH solution (Table 1). Similarly three distinct groups, dark yellow (6 varieties), light yellow (11 varieties) and no colour (6 varieties) were formed with the NaOH test. These two tests were greatly useful in resolving the groups formed by above three tests.

Thus the seed keys developed based on five chemical tests (phenol, modified phenol, FeSO_4 , KOH and NaOH tests) could clearly distinguish all the twenty three varieties from each other (Fig. 1). Initially phenol test has formed four major groups, which were later subdivided using other chemical tests. Phenol test in combination with modified phenol test resolved three varieties viz. Tadukan, Vijetha and Rathuheenathi and when both of them were used in conjunction with ferrous sulphate test could resolve further eight varieties viz., Varalu, Chenengi, ASD7, Swarna, PR113, QPE2, MTU 1004 and Pooja. The remaining twelve varieties were unlocked by using NaOH and KOH tests together. Further it is noticed that similar to FeSO_4 test, 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.

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 [8] 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 twenty three varieties individually. Hence these simple, rapid and reliable tests are of immense value for the varietal identification purpose in rice crop.

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