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# Identification of rice varieties on the basis of seed characteristics through various chemical tests

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## ABSTRACT

*An investigation was undertaken for identification of rice varieties on the basis of seed characteristics through various chemical tests. Seed samples of eleven rice cultivars (Subhadra, Sankar, Parijat, Suphala, Rudra, Kalinga-III, Khandagiri, Lalat, Bhuban, Sidhanta, Manaswini) which are under cultivation in the state of Odisha, were collected from Central Farm, Orissa University of Agriculture and Technology, Bhubaneswar and subjected to the following chemical tests viz., Phenol test, Modified phenol test ($FeSO_4$ and $CuSO_4$), NaOH test and KOH test. One control was also taken in case of NaOH test and KOH test. Based on phenol colour reaction, Sidhanta, Lalat, Subhadra showed dark brown colour, where as Parijat, Sankar, Khandagiri, Kalinga-III showed light brown colour and rest four varieties exhibited no colour change. The modified phenol test using $FeSO_4$ and $CuSO_4$ solution helped in further sub-division of standard phenol group. In case of $FeSO_4$ test, Parijat, Sankar, Rudra, Sidhanta, Bhuban showed dark brown colour and Kalinga-III, Khandagiri, Manaswini, Lalat, Subhadra, Suphala showed light brown colour. In case of $CuSO_4$ test, all the eleven varieties showed light brown colour. In case of NaOH test among eleven varieties Subhadra, Rudra, Khandagiri, Lalat, Sidhanta showed deep wine colour where as the other six varieties of rice showed wine colour. In case of KOH test except two varieties, Suphala and Khandagiri, other eight varieties showed deep wine colour. Control had no colour reaction in both NaOH and KOH test.*

***Key words:*** *Varietal identification, standard phenol, modified phenol, NaOH, KOH*

## INTRODUCTION

Crop varieties can be identified by studying the morphological, chemical and biochemical characteristics of seeds, seedlings and full grown plants. Though the study of morphological characteristics is relatively easy and cheap, they are not always fully accurate. The biochemical tests though accurate require a lot of expertise and special training to conduct and sometimes involve high cost. The study of characteristics of full grown plants is considered quite accurate, though it is season-specific and takes a long time to get the results. In contrast, the chemical tests can be used to reveal differences among the seeds and seedlings of different varieties and require virtually no technical expertise or training and can be completed in a relatively short time. The results of the chemical tests are distinct, quick, easy to carryout, reproducible and hence, these can be undertaken throughout the year under controlled laboratory conditions. As chemical reactions are based on chemical nature of protein, which are the exposed products of genes, chemical tests therefore are specific to genetic nature of the crop.

Several authors have investigated various chemical tests for varietal identification in different crop seeds. For identification of cotton genotypes, the chemical tests for seeds such as sodium hydroxide and potassium hydroxide were found to be useful, while gibberellic acid and 2,4-D soak tests for seedling response were found useful (Reddy et al., 2008). Vishwanath et al. (2013) conducted an investigation to study chemical tests for identification and characterization of 24 tomato cultivars *viz.*, standard

phenol test, modified phenol test, NaOH test, KOH test and seedling growth response to added chemicals. The study revealed that most of the cultivars studied were distinct from other cultivars. No single chemical test could distinguish all the varieties. However, distinguishable chemical characteristics were used to develop the keys for identification of each and every cultivar and all the cultivars were distinguished based on these identification keys. Kallihal et al. (2013) characterized the sunflower hybrid, KBSH-53 and its parental lines (CMS-335A and RHA-95-C-1) on the basis of biochemical tests at seed and seedling stages, *viz.,* potassium hydroxide (KOH), sodium hydroxide (NaOH) test and response of GA3 and 2,4-D at different concentrations on root and shoot growth and found differential response of the hybrid and its parents. Similar findings were also reported by Tiwari et al. (2013) also reported using phenol, modified phenol and NaOH tests of seeds and GA3 and 2,4-D tests of seedlings of four rice varieties. No individual chemical test was able to distinguish all the varieties but different chemical tests in conjunction were useful in varietal identification. Anitalakshmi et al. (2014) also reported that among various chemical tests, phenol and modified phenol tests gave the stable results and can be effectively used for cultivar differentiation in rice, as a routine test in seed testing laboratories. In contrast, Sripunitha et al. (2014) found that among several chemical tests, seedling response to KOH and NaOH, as also gibberellic acid, were found useful for examination of the red rice from white rice. Padma et al. (2015) found that KOH and $FeSO_4$ solutions are useful for identification of female parent (Sln1) of chilli hybrid CCH1 while NaOH test was useful to differentiate the chilli variety PKM1. Ukani et al. (2016) reported the use of phenol, peroxidase and NaOH tests for varietal identification in wheat, while KOH test proved ineffective. From the reports of several previous workers it is evident that there exists varietal as well as crop specific response to different chemical tests for establishing the genuineness of cultivars.

The present investigation was carried out to study the response of seeds of a few rice genotypes to various chemical tests, *viz.,* phenol test, modified phenol test (ferrous sulphate test and copper sulphate test), potassium hydroxide (KOH) test and sodium hydroxide (NaOH) test, to explore the possibility of using these tests for cultivar identification in rice.

## MATERIALS AND METHODS

Seed samples of eleven rice cultivars (Subhadra, Sankar, Parijat, Suphala, Rudra, Kalinga-III, Khandagiri, Lalat, Bhuban, Sidhanta, Manaswini), which are under cultivation in the state of Odisha, were collected from Central Farm, Orissa University of Agriculture and Technology, Bhubaneswar during *kharif* 2016 and subjected to the following chemical tests.

### Phenol test

Two hundred (50 x 4) seeds were soaked in distilled water for 16 hours at 25±1ºC. Then the soaked seeds were placed in petridishes lined with filter paper and moistened with 5 ml of 1% phenol solution and kept at room temperature (28º C) for 24 hours. Based on the intensity of colour, the seeds were classified into different categories, *viz.,* no colour change, light brown, brown, dark brown and black (Tiwari et al., 2013).

### Modified phenol test

Modified phenol test was conducted similar to standard phenol test except that seeds were soaked either in 15 ml of 0.5% $CuSO_4$ or 15 ml of 1% $FeSO_4$ solution for 24 hours instead of soaking seeds in distilled water. Then they were placed over a filter paper moistened with 4 ml of 1% phenol solution. The cultivars were classified based on colour reaction of seed coat into different colour classes such as no colour change, light brown, brown, dark brown, light grey, grey, dark grey (Vishwanath et al., 2013).

### Sodium hydroxide test

Four replications of 50 seeds of each cultivar were soaked in 15 ml of 5 % NaOH solution and kept at room temperature for 5 hours and thereafter, the change in colour of the solution was observed. Based on intensity of colour reaction, the genotypes were classified into three groups namely, no colour change, deep wine colour and wine colour (Sripunitha et al., 2014).

### Potassium hydroxide test

Two hundred seeds (50 x 4) were soaked in15 ml of KOH solution (5%) for 5 hours and after 5 hours change

in solution colour was observed and the cultivars were classified as no colour change, deep wine colour and wine colour (Sripunitha et al., 2014).

## RESULTS AND DISCUSSION

### Phenol test

The phenol colour reaction revealed that the eleven rice genotypes under investigation could be grouped into three colour groups. Out of eleven rice varieties, Sidhanta, Lalat, Bhuban and Subhadra showed dark brown colour, whereas Parijat, Sankar, Khandagiri and Kalinga-III showed light brown colour and rest three varieties, *viz.*, Suphala, Rudra and Manaswini exhibited brown colour change (Table1; Fig. 1). Phenol test is dependent on a flavonoid reaction in seed pericarp; colour change is due to reaction between vapour of phenol (and not as solution) and the glumes. Here, phenol gets oxidized into dark coloured melanin catalyzed by tryosinase enzyme. Similar results for phenol test were also reported by Anitalakshmi et al. (2004) and Sripunitha et al. (2014).

### Modified phenol test

The modified phenol test using $FeSO_4$ and $CuSO_4$ solution helped in further sub-division of standard phenol group. In case of $FeSO_4$ test, Parijat, Sankar, Rudra, Sidhanta and Bhuban showed dark brown colour, while Kalinga-III, Khandagiri, Manaswini, Lalat, Subhadra and Suphala showed light brown colour (Table 1 and Fig. 2). In case of $CuSO_4$ test, all the eleven varieties showed light brown colour (Table 1; Fig. 3).Similar results for modified phenol test was observed by Anitalakshmi et al. (2014) and Sripunitha et al. (2014).

**Table 1.** Response of seeds of 11 rice cultivars to different chemical tests

| Variety | Standard phenol test | Modified phenol test | | NaOH test | KOH test |
|---|---|---|---|---|---|
| | | $CuSO_4$ test | $FeSO_4$ test | | |
| Subhadra | Dark brown | Light brown | Light brown | Deep wine | Deep wine |
| Sankar | Light brown | Light brown | Dark brown | Wine | Deep wine |
| Parijat | Light brown | Light brown | Dark brown | Wine | Deep wine |
| Suphala | Brown | Light brown | Light brown | Wine | Wine |
| Rudra | Brown | Light brown | Dark brown | Deep wine | Deep wine |
| Kalinga-III | Light brown | Light brown | Light brown | Wine | Deep wine |
| Khandagiri | Light brown | Light brown | Light brown | Deep wine | Wine |
| Lalat | Dark brown | Light brown | Light brown | Deep wine | Deep wine |
| Bhuban | Dark brown | Light brown | Dark brown | Wine | Deep wine |
| Sidhanta | Dark brown | Light brown | Dark brown | Deep wine | Deep wine |
| Manaswini | Brown | Light brown | Light brown | Wine | Deep wine |


**Fig. 1.** Standard Phenol colour test

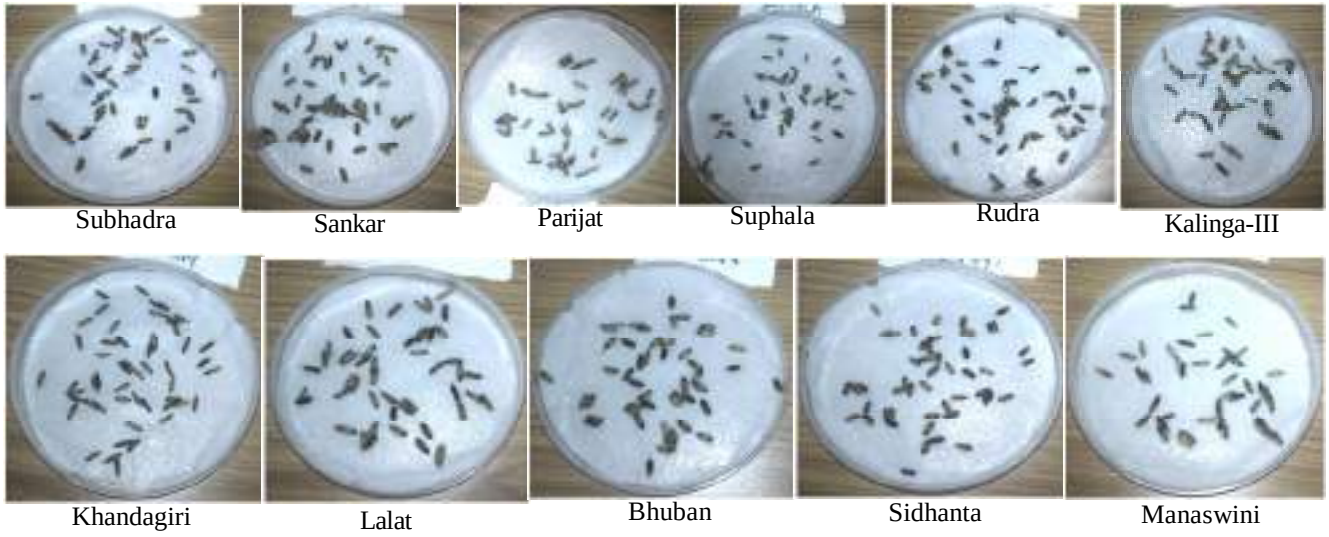


**Fig. 3.** Modified Phenol colour test with $CuSO_4$

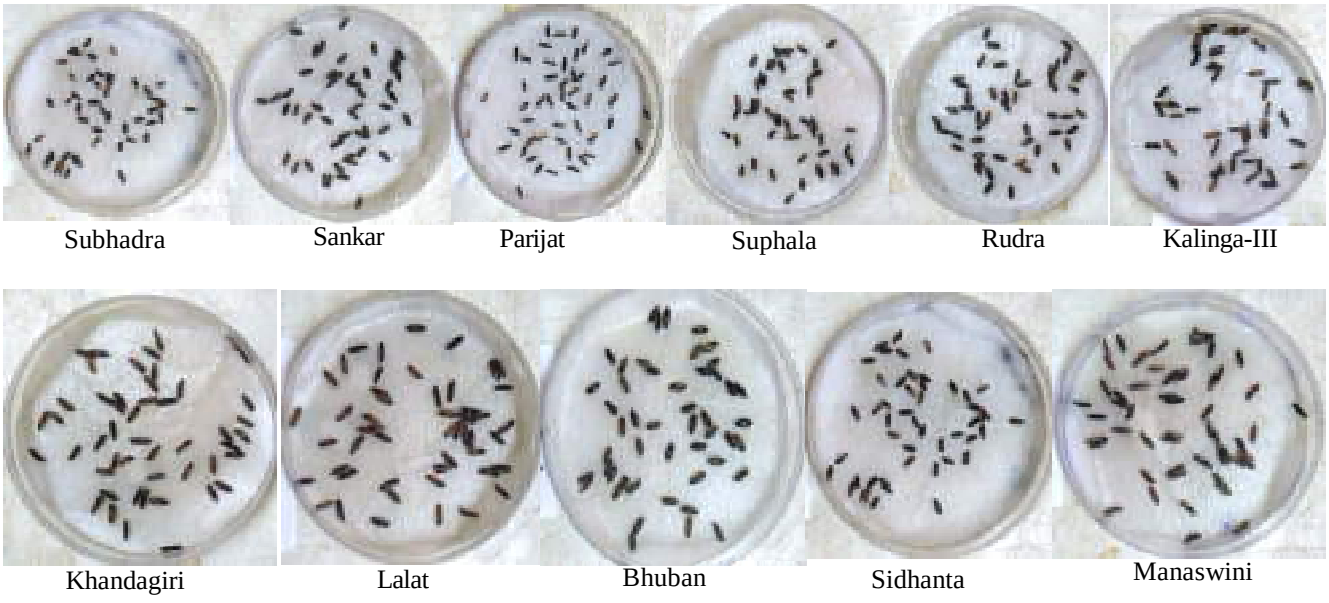


**Fig. 2.** Modified Phenol colour test with $FeSO_4$

## Sodium hydroxide test

Varied response of rice varieties to sodium hydroxide test was observed (Table1; Fig. 4). Based on the colour development of the decanted solution, the varieties were categorized into two groups, *viz.*, deep wine and wine colour. Among eleven varieties *Subhadra, Rudra, Khandagiri*, *Lalat* and *Sidhanta* showed deep wine colour where as the other six varieties of rice showed wine colour. Control had no colour reaction. Similar results were reported by Reddy et al., (2008) Vanangamudi et al., (1988).

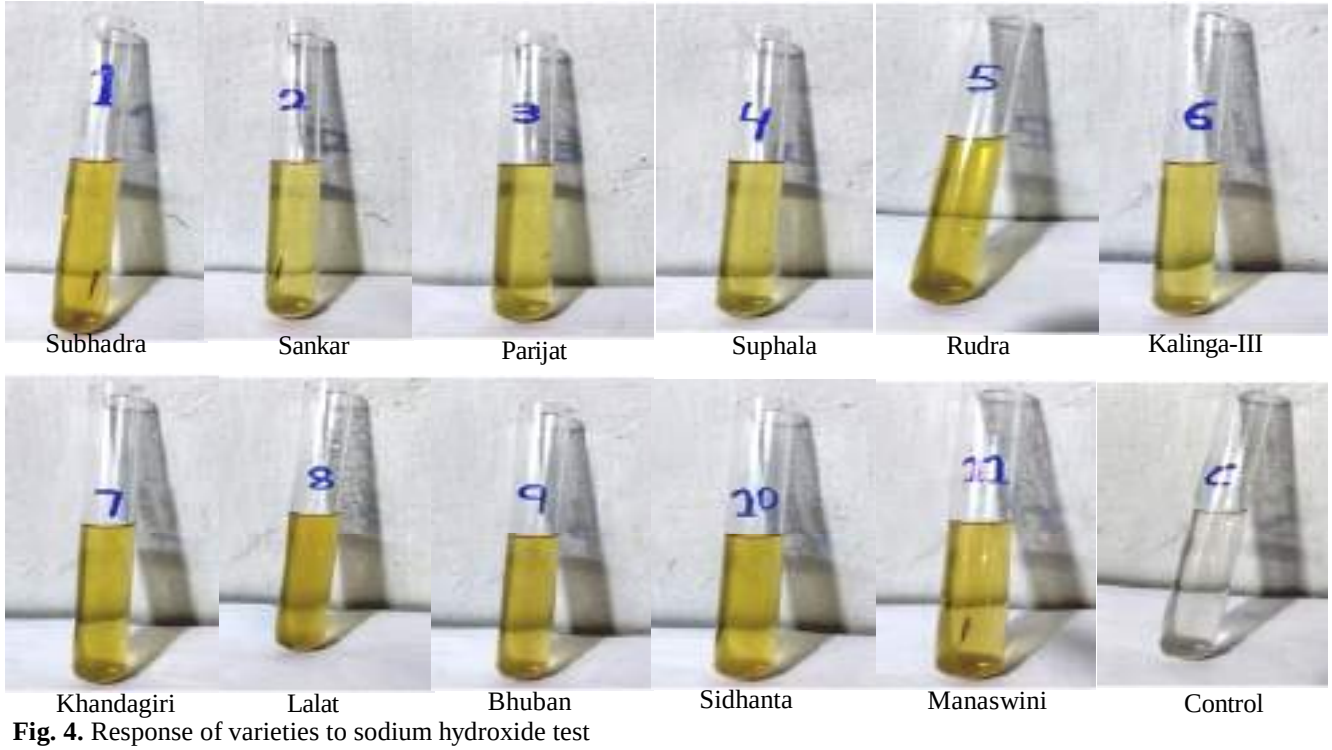


**Fig. 4.** Response of varieties to sodium hydroxide test

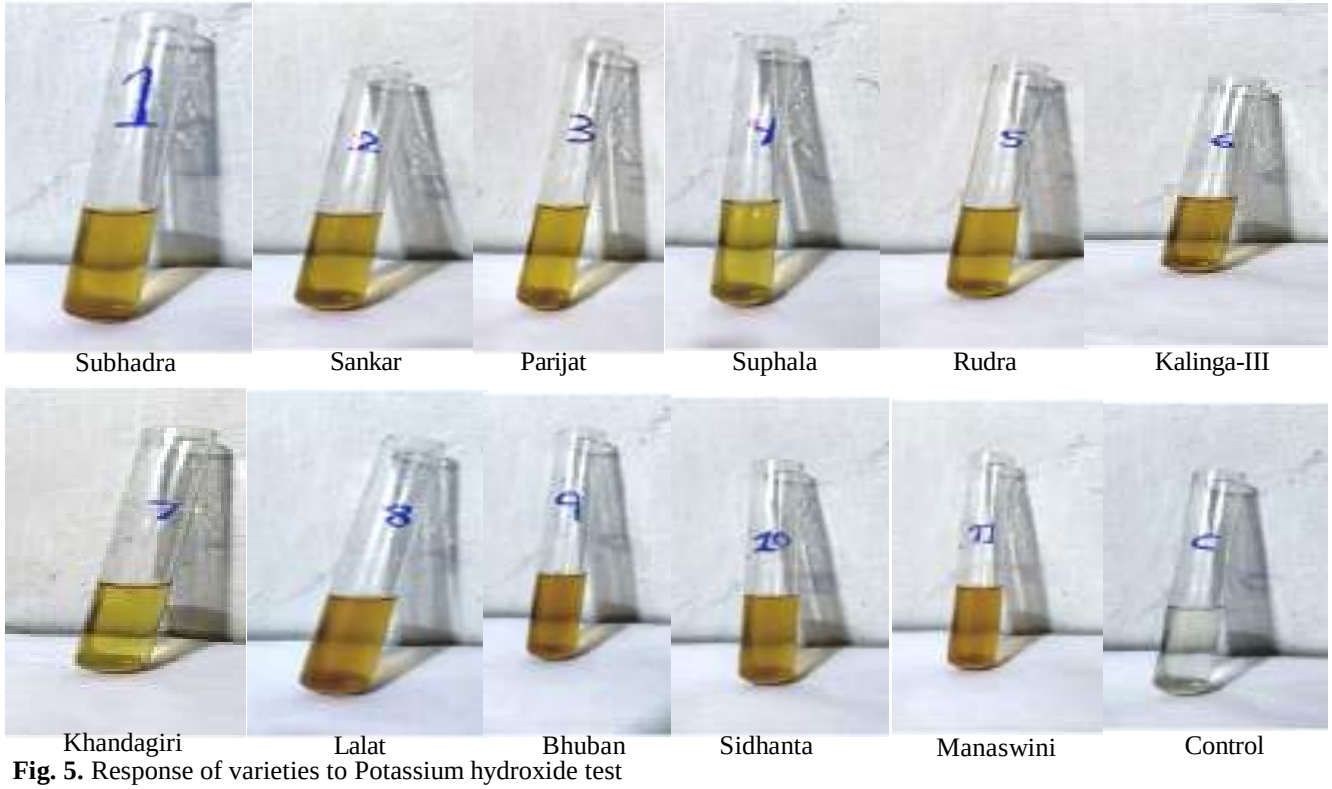


**Fig. 5.** Response of varieties to Potassium hydroxide test

### Potassium hydroxide test

Based on KOH test, cultivars were categorized into two groups, *i.e.*, deep wine colour and wine colour. Eight varieties showed deep wine colour except Suphala and Khandagiri (Table 1; Fig. 5). Control had no colour reaction. Similar results were reported by Reddy et al. (2008) Vanangamudi et al. (1988).

## CONCLUSION

Seed based techniques for varieties are warranted for rapid identification of seed lots. Therefore, in the present study, a strategy of using seed based chemical tests in development of seed keys for rice varieties. In view of highheritability and stability of phenol colour reaction, it could be used as primary diagnostic character fordistinguishing the rice genotypes. Therefore, it is suggested that the chemical tests could be used as simple, quick and cheap laboratory methods for varietal identification and characterization of rice cultivars.

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