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Research Article

VARIETAL RESPONSE TO VARIOUS CHEMICAL TESTS FOR THEIR CHARACTERIZATION IN RICE (*ORYZA SATIVA* L.)

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ABSTRACT
Eighteen rice genotypes were grouped on the basis of chemical tests (standard and modified phenol test, GA_3, 2,4-D and $KClO_3$). Among the chemical tests phenol and modified phenol tests gave the stable results and can be effectively used for cultivar differentiation. 100 ppm GA_3 and 2, 4-D gave more variation in shoot length among the genotypes compared to other concentrations. The study revealed that these tests could be effectively used for determining the varietal purity of rice for routine testing in seed testing laboratories as some of the cultivars showed distinct response to these chemical tests.

Key words: *Chemical Tests, Characterization, Phenol, KOH, 2,4-D, KClo3, Seed, Rice*

INTRODUCTION
The present trend of continuous release of rice varieties from Central and State Varietal Release Committee has warranted to develop suitable techniques for varietal identification at the laboratory level particularly when the seeds have been submitted for seed purity analysis. Maintenance of genetic purity of varieties is of primary importance for preventing varietal deterioration during successive regeneration cycles and for ensuring varietal performance at an expected level. The chemical tests reveal differences among the seeds and seedlings of different varieties. These tests 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 help in grouping of the genotypes. Therefore, an investigation was carried out to study the response of rice genotypes to various chemical tests to explore the possibility of using these tests for determination of cultivar purity in rice.

MATERIAL AND METHODS
Source of Seed:
Standard phenol test: One hundred (25 x 4) seeds were presoaked in distilled water for 24 hours at 25 $\pm$ 1°C. Then they were transferred on to two layers of Whatman No.1 filter paper saturated with four per cent phenol solution and incubated at 25 $\pm$ 1°C for 24 hours. Based on the intensity of colour they were classified as no change in colour (NC), light brown (LB), brown (BR) and dark brown (DB) according to Jaiswal and Agarwal (1995).
Modified phenol test: Modified phenol test was conducted similar to standard phenol test except soaking seeds in $CuSO_4$ (0.5 %) and $FeSO_4$ (1%) instead of distilled water. Then based on color reaction of seed coat the genotypes were classified as no change in colour (NC), light brown (LB), brown (BR), dark brown (DB) and black (BL).
KOH test: One hundred seeds (25X4) were soaked in KOH solution (4%) for three hours and change in solution colour was observed and the genotypes were classified as no change in colour (NC) and reddish brown (RB).
GA_3 test: One hundred seeds (25X4) were presoaked in 25, 50 and 100 ppm GA_3 for a period of 24 hours and germinated as per ISTA (1996). Observations were recorded on 14th day in terms of increase in shoot length over that of control.
2,4-D test: One hundred seeds (25X4) were soaked in 5, 10 and 15 ppm 2,4-D for a period of 24 hours and germinated as per ISTA (1996). Observations were recorded on 7th day in terms of decrease in shoot length over that of control.

$KClO_3$ test: Seedlings with 0.5 to 1.0 cm plumules were soaked in 0.1 per cent $KClO_3$ solution for 24 hours and were allowed to grow for 7 days and then the seedling injury was scored on a scale of 0 to 3. The per cent injury index was estimated using seedling injury index formula as prescribed by Yiwei (1993):

$$\text{Seedling injury index} = \frac{\Sigma \text{ (Score x seedling number)}}{\text{Highest score x total seedling number}}$$

Score:
0 : Normal growth without any injury
1 : Partial chlorosis at the margin of primary leaf
2 : Yellowing, partial rolling of primary leaf
3 : Whitening of whole seedling

RESULTS AND DISCUSSION

Out of eighteen varieties tested 16 of them were responded positively for standard phenol and modified phenol test with $CuSO_4$ (0.5%) and all the varieties responded positively to $FeSO_4$ (1.0%) test (Table 1). The varieties Mandya Vijaya and Prathibha showed brown staining for modified phenol test with $FeSO_4$ but these varieties failed to show the same response for standard phenol test and modified phenol test with $CuSO_4$. Only two varieties Red Triveni and Jyothi responded positively for KOH test showing reddish brown staining. The seed keys were developed using these parameters.

Table 1: Colour response of rice genotypes to various chemicals

Sl. No.	Genotypes	Standard phenol test	Modified phenol test $CuSO_4$ (0.5 %)	Modified phenol test $FeSO_4$ (1 %)	KOH test
1.	Aditya	BR	LB	LB	NC
2.	Heera	BR	LB	BR	NC
3.	IR-50	LB	LB	BR	NC
4.	Pusa-834	LB	LB	DB	NC
5.	Ratna	LB	LB	LB	NC
6.	Red Triveni	BR	LB	DB	RB
7.	IR-36	LB	LB	DB	NC
8.	Jyothi	LB	LB	LB	RB
9.	Kasturi	BR	LB	LB	NC
10.	Krishna Hamsa	LB	LB	LB	NC
11.	Rasi	LB	LB	BR	NC
12.	Vikas	BR	BR	DB	NC
13.	Intan	LB	DB	DB	NC
14.	Mahsuri	BR	BR	DB	NC
15.	Mandya Vijaya	NC	NC	BR	NC
16.	Prathibha	NC	NC	BR	NC
17.	Pusa Basmati-1	LB	LB	BR	NC
18.	Swarna	DB	BL	BL	NC

Note: NC- No colour change LB- Light Brown BR- Brown
DB- Dark Brown BL- Black RB- Wine Red

The use of phenol and KOH tests for varietal determination has been demonstrated by several workers in rice (Abrol and Uprety, 1972; Chauhan and Nanda, 1984; Vanangamudi *et al.*, 1988; Jaiswal and Agarwal, 1995).

The increase in shoot length over control due to application of GA_3 and reduction of shoot length due to application of 2,4-D varied significantly at all the concentrations. Shoot length was increased with increase in the concentration of GA_3 in all the genotypes but, the data on percent increase over control showed that 100 ppm GA_3 application gave more variation (35.10 to 113.61 %) among the genotypes. Similarly the study with 10 ppm 2, 4-D is helpful to differentiate the genotypes since more variation in percent decrease over control was observed among the tested genotypes. Similar results for GA_3 was observed by Goyal and Baijal (1980), Bansal *et al.*, (1992), Rohini Devi (2000). The differences in seedling growth might be due to differential ethylene production upon application of 2,4-D (Sundaru *et al.*, 1983).

Significant differences were observed between the genotypes for $KClO_3$ test. Cv. Swarna had showed least injury index (15) followed by Vikas (20). However, the genotype Aditya (73.33) with Heera, Pusa-834 (66.67) with Kasturi and Krishna Hamsa and Mahsuri (60.00) with Prathibha could not be differentiated as they recorded similar injury index. Similar response was also noticed by Rohini Devi (2000) in rice.

Among the chemical tests phenol and modified phenol tests gave stable results. In view of high heritability and stability of phenol colour reaction, it could be used as primary diagnostic character for distinguishing the rice genotypes. In case of growth substances 100 ppm GA_3 and 10 ppm 2, 4-D would be used for rice cultivar differentiation. Therefore it is suggested that the chemical tests could be used as simple, quick and cheap laboratory methods for determining the varietal purity of rice genotypes.

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