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Research Article

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Identification and characterization of popular rice (*Oryza sativa* L.) varieties through chemical tests

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ABSTRACT

Identification and characterization of crop varieties are crucial for ensuring the genetic purity of seeds. The present investigation was carried out to identify suitable chemical methods which are fast, reliable and easy for seed analysts, breeders and seed producers for identification of a variety. Twenty-five popular rice varieties in the seed supply chain of Tamil Nadu were subjected to phenol, modified phenol, NaOH, aroma, gelatinization temperature (alkali spreading value), GA_3 and 2,4-D tests. The results of the experiment revealed that phenol and modified phenol tests changed the colour of TKM 9 and TRY 1 variety to brown but no colour change was observed in the variety I.W. Ponni variety. The NaOH test is useful for identification of TKM 9 variety as it changed the colourless solution to red. GA_3 and 2,4-D tests characterized the varieties based on the shoot growth into two and three groups respectively. However, all the variety lacked aroma and exhibited high gelatinization temperature.

KEYWORDS: Rice, Chemical Test, Phenol Test, Sodium Hydroxide, GA_3, Varietal Identification

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INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food of Asia and life to about 90 per cent of rice is produced and consumed in here [1]. In order to cater to the demands of the ever-increasing population, a large number of paddy varieties have been released, mostly derived from crosses between high yielding varieties with common ancestors that have not only reduced the variability but also narrowed down the genetic base [2].

Increasing number of varieties could pose an issue of varietal identification which is extremely important to seed producers, seed analysts and in the registration of a variety under the Protection of Plant Varieties and Farmers' Rights Act (PPVFRA), 2001 [2,3]. Varietal identification is a crucial step in determining seed quality. It can be done through morphological, biochemical, molecular and chemical methods. The conventional approach is by using morphological traits which are highly influenced by the environment. Moreover, morphological traits alone may not be sufficient to distinguish the varieties [4]. Biochemical tests depend on plant ontogeny and cell constituents which vary with time and therefore their reliability is limited. Molecular methods are trustworthy but demand high technical knowledge, skill and huge investment for the establishment of the laboratory. In this context, varietal identification through chemical tests is gaining momentum.

Chemical tests *viz*., standard phenol test, modified phenol test, sodium hydroxide test, gelatinization temperature tests are simple, rapid results clearly visible, easy to interpret thus requires less technical knowledge [5,6]. These chemical tests can be utilized for various crops such as rice, wheat, sorghum, maize, cotton and red gram. Therefore, an investigation was carried out to ascertain the response of 25 commercial rice varieties to different chemical tests and to explore the possibilities of using these tests for varietal identification and characterization.

MATERIALS AND METHODS

Genetically pure seeds of twenty-five popular rice varieties in seed supply chain released by Tamil Nadu Agricultural University (TNAU) were obtained from Department of Rice and other Research Stations of TNAU (Table 1). The experiment was conducted at Department of Seed Science and Technology, TNAU, Coimbatore during 2019.

Table 1: List of 25 rice varieties utilized in the study

CO 51	ADT(R) 49
ADT 43	BPT 5204
ADT(R) 45	IR 20
ADT 36	CO 43
IR 50	CO(R) 50
ADT 37	CO 43 sub1
ASD 16	TRY 3
TPS 5	CR 1009
TKM 9	CR 1009 sub1
CB06803	TRY 1
CO 52	ADT 50
TKM 13	ADT 51
I.W.Ponni	

Standard Phenol Test

Twenty-five seeds of each variety were pre-soaked in double distilled water in petri dishes in three replications for 24 hours. Then the seeds were transferred to another petri plate containing 10 ml of 1 % phenol and kept undisturbed for 24 hours. The samples were scored based on the colour change as no colour change, light brown, brown, dark brown and black [2].

Modified Phenol Test

The protocol of this test is similar to that of standard phenol test but the seeds were pre-soaked in 10 ml of 0.5 % ferrous sulphate ($FeSo_4$) instead of double distilled water [2].

Sodium Hydroxide Test

Twenty-five seeds in each variety were soaked in 10 ml of 3 % NaOH solution in the petri dishes in three replications for three hours. The samples were scored based on the colour change of the NaOH solution to yellow and red [2].

Gelatinization Temperature Test

Three replications of ten decorticated seeds in each variety were soaked in a petri plate containing 10 ml of 1.7 % of Potassium Hydroxide (KOH) and the dishes were kept undisturbed at 30 °C for 24 hours. Gelatinization temperature is a mandatory test suggested by the Protection of Plant Varieties and Farmers' Rights Authority.

The response of kernels to the alkali were recorded on a seven-point scale as mentioned below [7].

Score	Depiction
1	Kernel not affected
2	Kernel swollen
3	Kernel swollen, collar incomplete and narrow
4	Kernel swollen, collar complete and wide
5	Kernel split or segmented, collar complete
6	Kernel dispersed, merging with collar
7	All kernel dispersed and intermingled

Aroma Test

Five grams of decorticated seeds were taken in a test tube containing 15 ml of double distilled water and allowed to soak for 10 minutes. The test tubes were then heated at 80°C for 15 minutes and the contents were transferred into the petri dishes which were cooled in the refrigerator for 20 minutes. Based on the smell the samples were scored as strongly scented, mild scented and non-scented.

Gibberellic Acid (GA_3) Test

Twenty-five seeds in each variety were soaked in 25 ppm gibberellic acid for 24 hours and were placed on the wet germination paper in two replications along with untreated control and rolled over such that the seeds do not spill over. The germination papers were placed in the germination room maintained at 25±2°C and 90±3 % relative humidity. The shoot length of ten random seedlings at the end of 14th day was measured and the percentage increase in the shoot length was worked out as given below [3]. Percentage decrease in the seedling length = Increment in shoot length of GA3 soaked seeds over control/seedling length of control×100.

2,4-D Test

Twenty-five seeds in each variety were soaked in 5 ppm 2,4-Dichlorophenoxyacetic acid for 24 hours were placed on the wet germination paper in two replications along with two replications of untreated control and rolled over. The germination papers were placed in the germination room maintained at 25±2°C and 90±3 % relative humidity. The shoot length of ten random seedlings at the end of 14th day was measured and the percentage decrease in the seedling length was worked out as given below [3]. Percentage decrease in the seedling length = Reduction in shoot length of 2,4 D soaked seeds over control/seedling length of control×100.

The varieties were grouped based on the percentage increase or decrease in seedling length as

1. Very low response - < 10 per cent
2. Low response - 10-30 per cent
3. Moderate response - > 30 per cent.

RESULTS AND DISCUSSION

Standard phenol test categorized the 25 rice genotypes in three classes as no colour change, light brown and brown colour with 1, 22 and 2 varieties respectively. Among the 25 varieties, no colour change was noticed in I.W. Ponni and brown colour change was observed in TKM 9 and TRY 1 variety, however, majority of the varieties were light brown (Figure 1). The results of the modified phenol test were same as that of phenol test with TKM 9 and TRY 1 variety showing brown colour and I.W. Ponni showing no colour change (Table 2). The colour change observed in the seeds might be due to polymerization and oxidation of phenol to melanin [8,9,10]. Similar kind of results were obtained by [2,3,11] in rice.

The varieties exhibited no variation for the gelatinization temperature and aroma test. All the varieties were non scented and exhibited high gelatinization temperature. Furthermore,

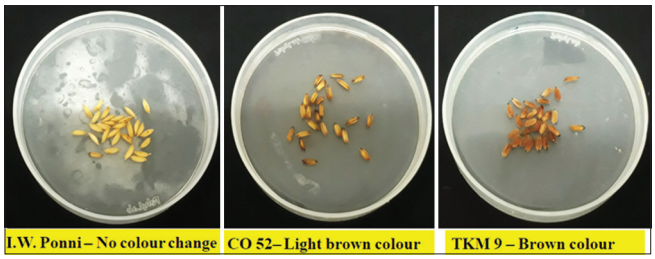

Figure 1: Response of rice varieties to Phenol test

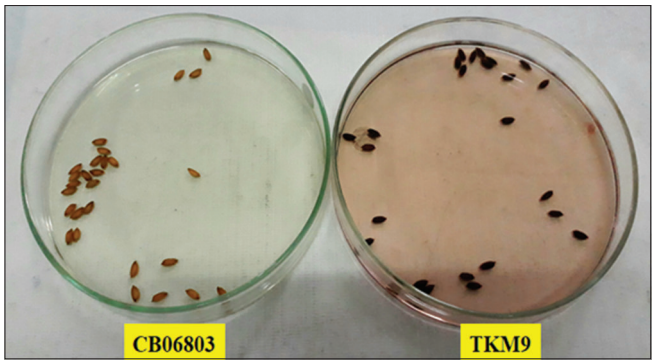

Figure 2: Response of rice varieties to Sodium hydroxide test

sodium hydroxide test categorised rice varieties into two groups based on the colour change as yellow and red (Figure 2). Only TKM 9, red rice variety changed the colour of NaOH solution to red whereas all other varieties to yellow colour (Table 2). The reasons for the colour change are not well understood however, [12] opined that pigment on the seed coat of red kernelled varieties would have influenced the colour change. The results are in concordance with the studies of [6,13,14]. The GA_3 test grouped the rice varieties into low and moderate response group with 2 (ASD 16 and BPT 5204) and 23 varieties respectively. The 2,4-D test was more significant in differentiating the varieties into different groups such as very low, low and moderate response with 7, 13 and 5 varieties in each group respectively (Table 3). Similar results were also observed by [5,6].

CONCLUSION

Among the chemical tests conducted phenol, modified phenol can be used for identification of TKM 9, TRY 3 and I.W. Ponni varieties. The NaOH test can be used exclusively for identification of TKM 9 variety. GA_3 and 2, 4-D tests characterized the varieties based on the shoot growth into two and three groups respectively. Thus, varietal identification and characterization through chemical tests are rapid and can be employed by breeders, seed analysts and seed producers.

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Table 2: Response of varieties to various chemical tests

Variety	Phenol test	Modified phenol test	Naoh test	Gelatinization temperature	Aroma test
CO 51	LB	LB	LY	KS	NS
ADT 43	LB	LB	LY	KS	NS
ADT 45	LB	LB	LY	KS	NS
ADT 36	LB	LB	LY	KS	NS
IR 50	LB	LB	LY	KS	NS
ADT 37	LB	LB	LY	KS	NS
ASD 16	LB	LB	LY	KS	NS
TPS 5	LB	LB	LY	KS	NS
TKM 9	B	B	R	KS	NS
CB06803	LB	LB	LY	KS	NS
CO 52	LB	LB	LY	KS	NS
TKM 12	LB	LB	LY	KS	NS
I.W. Ponni	NC	NC	LY	KS	NS
ADT 49	LB	LB	LY	KS	NS
BPT 5204	LB	LB	LY	KS	NS
IR 20	LB	LB	LY	KS	NS
CO 43	LB	LB	LY	KS	NS
CO(R) 50	LB	LB	LY	KS	NS
CO 43 sub1	LB	LB	LY	KS	NS
TRY 3	LB	LB	LY	KS	NS
CR 1009	LB	LB	LY	KS	NS
CR 1009 sub1	LB	LB	LY	KS	NS
TRY 1	B	B	LY	KS	NS
ADT(R) 50	LB	LB	LY	KS	NS
ADT 51	LB	LB	LY	KS	NS

*NS- Non scent KS- Kernel Swollen LB- Light Brown B- Brown NC- No colour change LY- Light Yellow R- Red

Table 3: Grouping of rice varieties based on shoot growth responses to GA_3 and 2,4-D test

Test	Response	Variety
GA_3	< 10	-
	10-30	ASD 16 and BPT 5204
	>30	CO 51, ADT 43, ADT 45, ADT 36, IR 50, ADT 37, TPS 5, TKM 9, CB06803, CO 52, TKM 12, I.W. Ponni, ADT 49, IR 20, CO 43, CO(R) 50, CO 43 sub1, TRY 3, CR 1009, CR 1009 sub1, TRY 1, ADT(R) 50 and ADT 51
2, 4-D	< 10	IR 50, TPS 5, CB06803, I.W. Ponni, ADT 49, CO(R) 50 and CR 1009
	10-30	CO 51, ADT 43, ADT 45, ADT 36, ADT 37, ASD 16, TKM 9, CO 52, TKM 12, IR 20, CO 43, CO 43 sub1 and TRY 3
	>30	BPT 5204, CR 1009 sub1, TRY 1, ADT(R) 50 and ADT 51

REFERENCES

1. Bandumula N. Rice production in Asia: Key to global food security. Biological Sciences. 2018; 88(4): 1323-1328.
2. Vijayalakshmi B, Vijay D. Development of seed keys for varietal identification in Rice (*Oryza sativa* L.) using chemical tests. Seed Research. 2009; 37: 56-61.
3. Rao PS, Bharathi M, Reddy KB, Keshavulu K, Rao LS, Neeraja CN. Varietal identification in rice (*Oryza sativa*) through chemical tests and gel electrophoresis of soluble seed proteins. Indian Journal of Agricultural Sciences. 2012; 82(4): 304-311.
4. Vishwanath K, Pallavi HM, Nethra N, Prasad RS. Chemical tests for identification and characterization of tomato cultivars. Plant Breeding and Seed Science. 2013; 68: 3-12.
5. Anitalakshmi V, Gowda R, Sathisha CS, Prasad R. Varietal response to various chemical tests for their characterization in rice (*Oryza sativa* L.). Indian Journal of Plant Sciences. 2014; 3(2): 177-179.
6. Sripunitha A, Sivasubramaniam K. Varietal characterization rice

varieties based on chemical methods. Trends in Bioscience. 2014; 7(20): 3139-3146.
7. Little RR, Hilder GB, Dawson EH. Differential effect of dilute alkali on 25 Varieties of milled white rice. Cereal Chemistry. 1958; 35: 111-126.
8. Britton G. The biochemistry of natural pigments. Cambridge: Cambridge University Press; 1985.
9. Solano F. Melanins: skin pigments and much more-types, structural models, biological functions, and formation routes. New Journal of Science, 2014; 2014:1-28.
10. Glagoleva AY, Shoeva OY, Khlestkina EK. Melanin Pigment in Plants: Current Knowledge and Future Perspectives. Frontiers in Plant Science, 2020; 11: 770.
11. Tiwari JK, Rastogi N, Chandrakar P, Sarawgi A, and Verulkar S. Identification of rice varieties through chemical tests. Indian Journal of Plant Science. 2013; 3: 177-179.
12. Ram MS, Dowell FE, Seitz L, Lookhart G. Development of standard procedures for a simple, rapid test to determine wheat color class. Cereal chemistry. 2002; 79(2): 230-237.
13. Masuthi D, Vyakarnahal BS, Deshpande VK, Jagadeesha RC, Mukesh LC, Khaja Rubina S. Characterization of traditional aromatic rice cultivars by chemical markers. International Journal of Advanced Research. 2015; 3(5): 103-107.
14. Singh C, Sripathy KV, Jeevan KSP, Naik K, Pal G, Bhaskar K, Ramesh KV, Somasundaram G. Delineation of inheritance pattern of aleurone layer colour through chemical tests in rice. Rice, 2017; 10(1): 1-10.