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Characterization and identification of rice landraces through chemical tests

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Abstract
Varietal characterization and identification are very important for maintaining genetic purity of the varieties during seed production, maintenance and conservation. Since now-a-days traditional rice varieties are gaining momentum, the study on varietal characterization were carried out in rice seeds using different chemical tests. In which, 50 varieties of rice landraces were characterized using phenol, modified phenol, ferrous sulphate, sodium hydroxide and potassium hydroxide tests. The results indicated that the phenol and modified phenol tests showed more colour variations *viz*., no colour, light brown, brown, dark brown and black. The varieties such as Kattuyaanam, Poovan samba and Sorna masuri showed greater response to phenol test with dark brown colour and the varieties *viz*., Karunkuruvai, Idly and Kullankar changed to black colour. Modified phenol test was used for further subgrouping of varieties which showed different colour reactions in some varieties *viz.,* Navara, Karukka, Kuzhiyadichan etc. Ferrous sulphate test classifies the varieties into four groups *viz.,* no colour, dark grey streaks, brown streaks and brown spots. Varieties *viz*., Thulasi vaasanai, Sivan samba and Sorna had dark grey streaks; Nootripathu, Kothamalli samba and Seeraga samba showed brown streaks; and Norungan, Mysore malli, Poovan samba showed brown spots. Similarly, NaOH and KOH tests grouped the varieties into four categories namely light yellow, yellow, pale red and red and the varieties like Salem sanna, Illuppaipoo samba and Sivan samba showed yellow colour and Kothamalli samba, Nootripathu and Poongar recorded with red colour. Also, the NaOH and KOH tests can be used very well for distinguishing the red rice varieties from white rice varieties. Therefore, a complementary set of these chemical tests can be used for varietal identification of landraces.

Keywords: Traditional rice varieties, chemical tests, colour change, varietal identification

Introduction
Rice is the food source for people in many parts of the world especially Asia and Sub-Saharan Africa. It is cultivated over an area of 162.06 million hectares with the production of 700 million tonnes of raw rice and 470 million tonnes of milled rice [1]. Also, it is the most consumed crop in the world which accounts about 108 million tonnes [2]. India is the second largest producer of rice in the world which accounts an area of 43.8 million hectares and production of 101.98 million tonnes during 2019-2020. The average productivity of rice in India is 2,700 kg/ha [3]. China is the leading country with more rice production and export followed by India, Bangladesh and Indonesia.

Traditional rice varieties are gaining importance in the last decade and it is cultivated by a large number of farmers. They are noted for their health benefits and the ability to withstand environmental stresses. Also, resistant hybrids for stress conditions were developed by transferring genes from various land races. In addition, the landraces possess high nutritional value and are used to cure diabetes, blood pressure and obesity. They also contain anti carcinogenic properties [4], anti-neurodegenerative activity [5] and contain powerful antioxidants such as tocopherols, tocotrienols, polyphenols, flavonoids, oryzenols and vitamin C which also plays a vital role for protection against chronic diseases [6] and scavenging activity more than the hybrids and white rice varieties [7].

With increased awareness among the people, the need to supply good quality seed increases and the best way to maintain seed quality is by maintaining the genetic purity. The first step in maintaining genetic purity is varietal identification that can be done by many ways *viz*., morphological, chemical and molecular methods. Conventional method of varietal identification through grow out test takes more time which also dependent on environment factors and therefore, this alone is not enough to identify the varieties [8]. Molecular methods have been used to characterize the varieties as they are independent of environment [9-11]. However, these methods require high skill and are more expensive.

Biochemical methods depend highly on the cell constituents and plant development and it varies with time [8]. While, chemical methods have been used to identify the varieties and the results can be obtained in a fair short span of time. Chemical tests such as Phenol, modified phenol, NaOH and KOH tests have been used to characterize 155 rice germplasm accessions [19]. Mathad *et al*, [20] used ferrous sulphate test for determining genetic purity in pigeon pea. Similarly, NaOH and KOH tests were used for identification of white and red rice cultivars [14, 17-19]. Therefore, an experiment was conducted to characterize and identify 50 traditional rice varieties using chemical tests.

Materials and Methods

Collection of seeds

Seeds of 50 traditional rice varieties as listed in table 1 were collected from farmer's holdings of various parts of Tamil Nadu *viz*., Cuddalore, Thanjavur, Nagapattinam, Tirunelveli, Madurai and Kanyakumari districts. The experiment was conducted in the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore, India during 2019-21. The collected seeds were multiplied and purified in the field by removing the off-type plants based on plant and seed morphological traits. Then, the genetically pure seeds were used for characterization along with the basic seed materials collected from farmers for identification of other distinguishable varietal seeds. The chemical tests *viz*., phenol, modified phenol, ferrous sulphate, sodium hydroxide and potassium hydroxide tests were used to characterize the seeds.

Phenol test

Twenty-five seeds of each variety in three replications were presoaked in Petriplates containing double distilled water for 24 h and the presoaked seeds were transferred to Petriplates containing Whatman No. 1 filter paper saturated with 1% phenol solution for 24 h. After that, the change in seed testa and glume colour was recorded and the varieties were grouped into light brown, brown, dark brown and black. Royal Horticultural Society (RHS) colour chart was used for further classification of varieties based on colour intensity. In which, light brown colour was grouped into N199D grey brown group, brown into 200D brown group, dark brown into 200A and 200B, black into 202A black [14].

Modified phenol test

Twenty-five seeds of each variety in three replications were presoaked in 0.5% $FeSO_4$ for 24 h and then, the seeds were soaked in 1% phenol solution for another 24 h. After completion of the test period, change in testa colour was recorded as per the procedure followed in phenol test [14].

Sodium hydroxide (NaOH) test

Twenty-five seeds of each variety in three replications were soaked in 10 ml of 5% NaOH solution for 1 h in test tubes at room temperature. Then, the seeds were grouped into different categories based on the change in NaOH solution to light yellow, yellow, pale red and red [14].

Potassium hydroxide (KOH) test

Twenty-five seeds of each land races in three replications were soaked in test tubes containing 10 ml of 5% KOH solution for 1 h at room temperature. Then, the seeds were classified based on the change in colour of the solution into light yellow, yellow, pale red and red [19].

Ferrous sulphate ($FeSO_4$) test

Seeds of landraces were soaked in 1.5% $FeSO_4$ for 4 h under room temperature. Then, the excess moisture was removed and the change in seed testa colour was recorded as dark grey streaks, brown streaks and brown spots [20].

Statistical analysis

The varieties that are mentioned in Table 1 were analyzed using cluster analysis in Minitab applying ward's method which groups the varieties using Euclidean distance in similarity matrix [21].

Results and Discussion

Characterization traditional rice varieties by chemical tests showed that the phenol test categorized the 50 landraces into five major groups based on colour reaction of the testa such as no colour change, light brown (RHS: N199A), brown (RHS: 200 C & D), dark brown (RHS: 200 A & B) and black colour (RHS: 202 A). Among the 50 varieties, no colour change was noticed in 10 varieties, light brown colour in 8 varieties, brown in 13 varieties, dark brown in 15 varieties and black in 4 varieties (Table 1). Majority of varieties *viz*., Kattuyaanam, Chithiraikar, Vaasanai seeraga samba and Mysore malli exhibited dark brown colour followed by Mattaikar, Samba Mosanam, Nootripathu, Kothamalli samba with brown colour. Black colour was noticed in Karunkuruvai, Idly and kullankar (Figure 1a). Similarly, the modified phenol test has showed colour variations same as that of phenol test except 13 varieties *viz*., Seeraga samba, Sorna, Poongar, Vellaikavuni, Milagu samba, Navara, Karukka, Vaasanai seeraga samba, Kuzhiyadichan, Paalthondi and Mottakkur which showed different colours than the phenol test (Figure 1b). Similar kind of results due to chemical tests for varietal identification was obtained earlier by many workers in rice [14, 16-19, 21-23].

Table 1: Effect of chemical tests on variations in seed colour of traditional rice varieties.

S. No.	Varieties	Phenol test	Modified phenol test	$FeSO_4$ test	NaOH test	KOH test
1.	Thulasi vasanai	No colour	No colour	Dark grey	Light yellow	Light yellow
2.	Salem sanna	No colour	No colour	Brown streaks	Yellow	Yellow
3.	Thanga samba	No colour	No colour	Dark grey	Yellow	Light yellow
4.	Illuppaipoo samba	No colour	No colour	No colour	Yellow	Yellow
5.	Sivan samba	No colour	No colour	Dark grey	Yellow	Light yellow
6.	Kaalabadh	No colour	No colour	No colour	Yellow	Yellow
7.	Sembuli samba	No colour	No colour	Brown streaks	Yellow	Light yellow
8.	Seeraga samba	No colour	Brown	Brown streaks	Light yellow	Light yellow
9.	Kudhiraivaali samba	No colour	No colour	Dark grey	Pale red	Pale red
10.	Kallundrikar	Brown	Brown	Brown streaks	Red	Yellow
11.	Sorna	Brown	Light brown	Dark grey	Light yellow	Light yellow
12.	Kothamalli samba	Brown	Brown	Brown streaks	Red	Red
13.	Nootripathu	Brown	Brown	Brown streaks	Red	Red

14.	Poongar	Brown	Dark brown	Brown streaks	Red	Pale red
15.	Samba mosanam	Brown	Brown	Brown spots	Light yellow	Red
16.	Maranel	Brown	Brown	Dark grey	Light yellow	Light yellow
17.	Kallurundaikar	Brown	Brown	Brown streaks	Light yellow	Pale red
18.	Thengaipoo samba	Brown	Brown	Dark grey	Light yellow	Light yellow
19.	Kallukar	Light brown	Light brown	Dark grey	Yellow	Light yellow
20.	Vellai kavuni	Light brown	Brown	Brown streaks	Light yellow	Light yellow
21.	Anaikomban	Light brown	Light brown	Dark grey	Pale red	Pale red
22.	Rajamannar	Light brown	Light brown	Dark grey	Red	Red
23.	Kalanamak	Light brown	Light brown	Dark grey	Red	Pale red
24.	Poovan samba	Dark brown	Dark brown	Brown spots	Red	Pale red
25.	Mappillai samba	Brown	Brown	Brown streaks	Red	Yellow
26.	Kattuyanam	Dark brown	Dark brown	Brown streaks	Red	Red
27.	Milagu samba	Dark brown	Black	Dark grey	Yellow	Light yellow
28.	Kattanur	Dark brown	Brown	Dark grey	Pale red	Pale red
29.	Kuruvai kalangium	Dark brown	Brown	Brown streaks	Red	Red
30.	Sorna masuri	Dark brown	Dark brown	Brown streaks	Pale red	Light yellow
31.	Navara	Dark brown	Black	Dark grey	Yellow	Yellow
32.	Karukka	Dark brown	Brown	Brown streaks	Red	Pale red
33.	Vaasanai seeraga samba	Dark brown	Brown	Brown streaks	Red	Pale red
34.	Mysore malli	Dark brown	Brown	Brown spots	Yellow	Pale red
35.	Kuzhiyadichan	Dark brown	Brown	Brown streaks	Pale red	Red
36.	Norungan	Dark brown	Dark brown	Brown spots	Pale red	Light yellow
37.	Chithiraikar	Dark brown	Dark brown	Brown streaks	Red	Red
38.	Paalthondi	Dark brown	Brown	Dark grey	Red	Red
39.	Mattaikar	Brown	Brown	Dark grey	Red	Red
40.	Mottakur	Brown	Light brown	Dark grey	Red	Red
41.	Karunkuruvai	Black	Black	No colour	Red	Red
42.	Karuppu kavuni	Black	Black	No colour	Red	Red
43.	Idly	Black	Black	No colour	PR	Red
44.	Kullankar	Black	Black	No colour	Red	Red
45.	Muthuvellai	Dark brown	Dark brown	Brown spots	Light yellow	Light yellow
46.	Vadakathi samba	Brown	Brown	Dark grey	Red	Light yellow
47.	Ponmani samba	Light brown	Light brown	Dark grey	Light yellow	Light yellow
48.	Rangoon samba	Dark brown	Dark brown	No colour	Red	Light yellow
49.	Manjal ponni	Light brown	Light brown	Dark grey	Yellow	Yellow
50.	Thooyamalli	No colour	No colour	Dark grey	Light yellow	Light yellow

Fig 1a: Phenol test

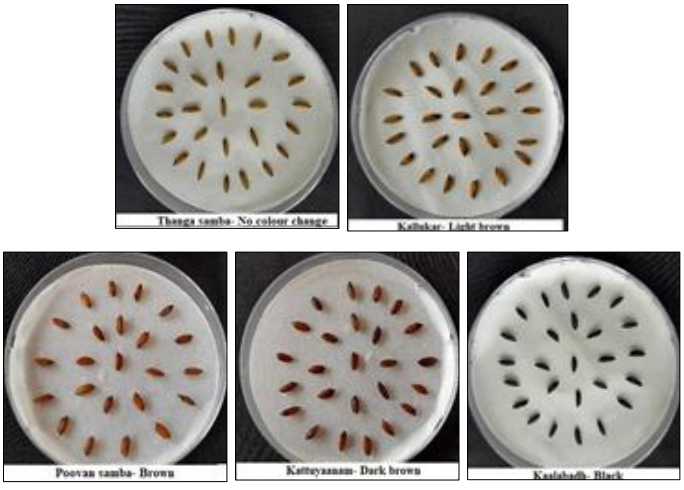
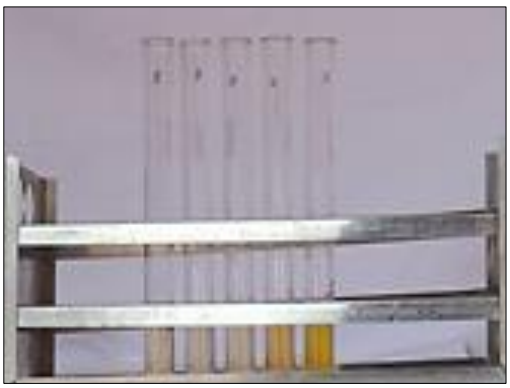


Fig 1b: Modified phenol test

Fig 1c: Ferrous sulphate test



5. Thulasi vaasanai
3. Seeraga samba
13. Vellaikavuni
16. Illupaipoo samba
1. Salem sanna

Fig 1d: NaOH test

11. Mottakur
10. Kuruvai kalanjium
8. Kallundrikar
7. Poongar
17. Vaasanai seeraga samba
12. Nootripathu
15. Kullankar
2. Idly
4. Karunkuruvai

Fig 1e: KOH test

6. Sorna
1. Bhavani
4. Tulasi vaasanai
19. Thanga samba
11. Illupaipoo samba
7. Salem sanna

18. Idly
20. Karunkuruvai
2. Mysore malli
3. Karukka
9. Sorna masuri
10. Anaikomban
16. Vaasanai seeraga samba

8. Poongar
5. Mottakur
14. Kothamalli samba
17. Kullankar
12. Nootripathu
13. Kuruvai kalanjium
15. Kattanur

Fig 1: Response of traditional rice varieties to different chemical tests.

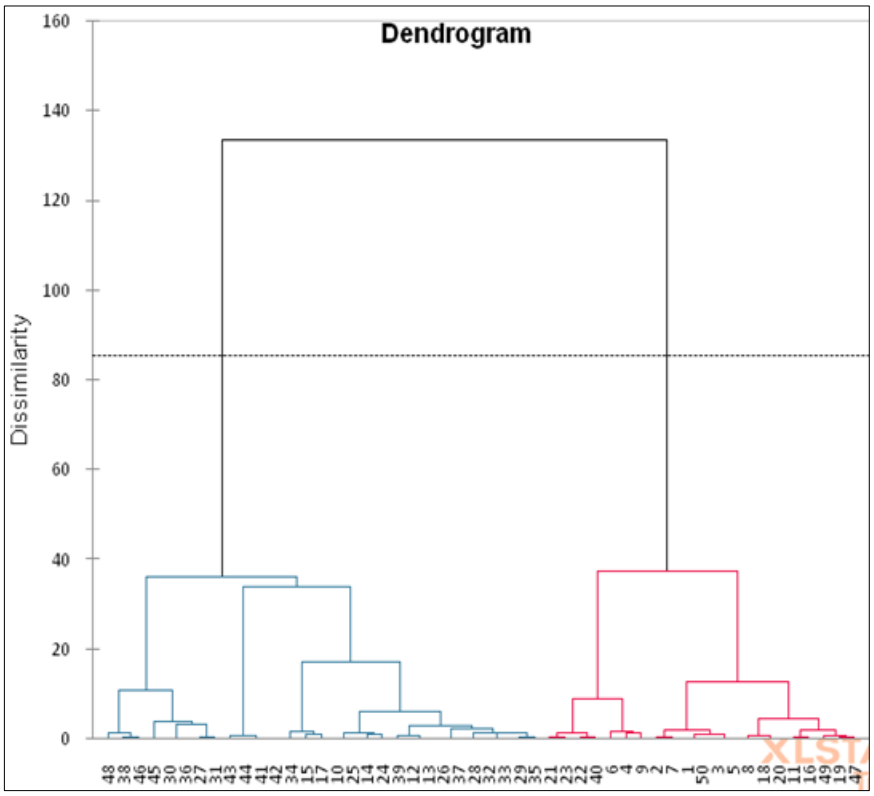


Fig 2: Dendrogram shows clustering of varieties at 86% dissimilarity matrix.

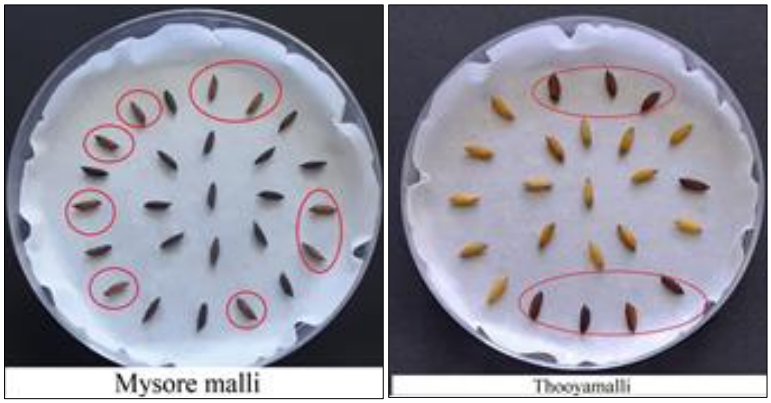


Fig 3: Identification of off-types using phenol test.

Phenol test is an index of polyphenol activity and it is associated with intra-varietal diversity. The melanin is formed by the oxidation of phenol via orthoquinones and hydroquinones and is controlled by single gene localized in the seed coat [16]. The aleurone layer is also reported to be involved in the synthesis of oxidase enzymes like laccase, tyrosinase, monophenol oxidase, polyphenol oxidase and horse-radish peroxidase, which catalyzes to form a colour reaction [24, 25]. Among them, polyphenol oxidase (PPO) is one of the enzymes that are involved in oxidation of phenol and formation of brown coloured pigment called melanin [26, 27]. Polyphenol oxidases avail molecular oxygen, which undergoes hydroxylation and dehydrogenation of phenolic compounds to form reactive o-quinones. These o-quinones

alkylate nucleophilic groups and self-polymerize to form melanin polymers [28].

In addition, the colour formation in aleurone layer occurs by phenol oxidation in two reactions. In the first reaction, the aromatic ring of phenol can be hydroxylated to form catechols or quinols followed by the second reaction where the quinols or catechols undergo oxidation to form quinones [29]. Two major genes and their allelic interactions control this reaction, which is localized in rice seed aleurone layer. The ability of genotype to form colour depends on the tyrosinase activity, which is located at aleuronic layer [30]. Qian *et al.* [31] reported that major QTL (qPH-4a) *i.e.* responsible for phenol colour reaction is located on chromosome 4, which showed the total phenotypic variation as high as 94.6%. Also, two minor QTLs (qPH-1 and qPH-4b) located on chromosome 1 and 4 have account of total phenotypic variation of 14.9 and 29.5%, respectively.

Ferrous sulphate test classifies the landraces into four groups as no colour change, presence of dark grey streaks, brown streaks and brown spots on the seed coat. The results indicated that 7 varieties showed no colour change, 20 varieties had dark grey streaks, 19 varieties with brown streaks and 4 varieties with brown spots. The varieties *viz*., Sorna masuri, Navara, Thooya malli, Samba mosanam and Mappillai samba showed greater response by forming brown streaks and brown spots (Figure 1c). Similar results were obtained in rice [14, 23] and pigeon pea [20]. The presence of an enzyme system which causes colour change was utilized in rice by many workers in which, Saharan [14] have categorized 33 genotypes and Nagendra *et al.* [21] characterized 23 varieties. Similarly, Mathad *et al.* [20] studied the genetic purity determination in pigeon pea by using $FeSO_4$ test.

Sodium hydroxide test classified the varieties into four groups *viz*. light yellow, yellow, pale red and red. Of these varieties, 11 varieties showed the colour change in the solution into light yellow, another 11 varieties with yellow, 7 varieties with pale red and remaining 21 varieties with red colour. Similar results were also observed by workers in rice varieties [14, 17-19, 21-23]. Likewise, KOH test categorized the landraces into four groups as that of NaOH test (Table 1). In which, 19 varieties showed light yellow, 6 varieties displayed yellow colour, 10 varieties showed pale red and the remaining 15 varieties changed to wine red. The results are in accordance with the earlier works carried out in rice [14, 17-19, 21-23, 32-33]. The colour change of solution may be influenced by the presence of pigments in red and brown kernelled varieties [21]. Thus, the red rice varieties can be easily identified from white rice varieties using NaOH and KOH tests. In this regard, the varieties *viz*, Karuppu kavuni, Kothamalli samba, Karunkuruvai, Nootripathu, Kattuyanam, Mattaikar, Mottakkur, Idly and Kullankar showed red colour and can easily be identified using NaOH and KOH tests (Figure 1d & 1e).

The results of the cluster analysis of traditional varieties for various chemical tests showed that there were two major clusters formed at 86% dissimilarity matrix. In which, cluster I contains 21 varieties and cluster II contains 29 varieties (Figure 2). Therefore, the varieties in a same cluster behave in a similar way to chemical tests and shows less variation among themselves and may be closely related. Similar kind of cluster analysis for grouping of rice varieties was also done in improved varieties [21].

The results of the cluster analysis of traditional varieties for various chemical tests showed that there were three major clusters formed at 47% similarity matrix. In which, cluster I contains 9 varieties and cluster II contains 12 varieties and cluster III groups 29 varieties (Table 1) (Figure 2). The inter cluster distance is maximum between cluster I and III (4.29628) followed by distance between cluster II and III (3.04418) and between I and II (2.61215). Therefore, the varieties in a same cluster behave in a similar way to chemical tests and shows less variation among themselves and may be closely related. The genotypes within a cluster show less variation between themselves than with other genotypes in another cluster. Similar kind of cluster analysis for grouping of rice varieties was also done in improved varieties [21].

Conclusion

Fifty traditional rice varieties were characterized using chemical tests such as phenol, modified phenol, ferrous sulphate, NaOH and KOH tests and the results showed that individual chemical tests have limited value and when used in a complementary series or in conjunction, these tests can separate any number of varieties [33]. Moreover, these chemical tests are stable, rapid, easy and reliable while going for varietal identification and characterization in traditional rice varieties.

References

1. Shahbandeh M. World rice acreage 2010-2019 [Internet]. Statista C2007-2021. [Cited 2021 September 4]. https://www.statista.com/statistics/271969/world-rice-acreage-since-2008/
2. Rice productivity [Internet]. Rice productivity C2013. [Cited 2020 September 4]. https://ricepedia.org/rice-as-a-crop/rice-productivity
3. The Economic times.org [Internet]. Record rice production likely to propel kharif output to a new high C2020. [Cited 2021 September 4]. https://economictimes.indiatimes.com/news/economy/agriculture/record-rice-production-likely-to-propel-kharif-output-to-a-new-high/articleshow/78289616.cms
4. Shipp J, Abdel-Aal ES. Food applications and physiological effects of anthocyanins as functional food ingredients. The open food science journal 2010;4(1).
5. Kim MK, Kim HA, Koh K, Kim HS, Lee YS, Kim YH. Identification and quantification of anthocyanin pigments in colored rice. Nutrition Research and Practice 2008;2(1):46-9.
6. Tsuda T, Watanabe M, Ohshima K, Yamamoto A, Kawakishi S, Osawa T. Antioxidative components isolated from the seed of tamarind (*Tamarindus indica* L.). Journal of Agricultural and Food Chemistry 1994;42(12):2671-4.
7. Oki T, Masuda M, Nagai S, Take'ichi M, Kobayashi M, Nishiba *et al*. Radical-scavenging activity of red and black rice. In Rice is life: scientific perspectives for the 21st century. Proceedings of the World Rice Research Conference held in Tsukuba, Japan. International Rice Research Institute (IRRI) 2004 2005, 256-259.
8. Vishwanath K, Pallavi HM, Nethra N, Prasad SR. Chemical tests for identification and characterization of tomato cultivars. Plant Breeding and Seed Science 2013;68:3-13.
9. Tanksley SD, Ganal MW, Prince JP, De Vicente MC, Bonierbale MW, Broun P *et al*. High-density molecular linkage maps of the tomato and potato genomes. Genetics 1992;132(4):1141-60.

10. Rajendra Prasad S, Prasanna KP. Varietal characterization of tomato cultivars based on RAPD markers. Research Journal of Agriculture and Biological Sciences 2010;6(6):713-5.
11. Lucchese C, Dinelli G, Miggiano A. Identification of pepper (*Capsicum* spp.) cultivars by field and electrophoresis tests 1999.
12. Padma J, Sivasubramaniam K, Anbu S. Identification of chilli genotypes through chemical tests. Journal of Academia and Industrial Research (JAIR) 2015;4(5):152-4.
13. Thangavel P, Bharathi A, Natarajan N, Evera T. Varietal grouping in sorghum by seed and seedling morphology and response to chemical testing. Karnataka Journal of Agricultural Sciences 2010;18(3).
14. Vijayalakshmi B, Vijay D. Development of seed keys for varietal identification in Rice (*Oryza sativa* L.) using chemical tests. Seed Res 2009;37(1&2):56-61.
15. Singh C, Sripathy KV, SP JK, Pal G, Ramesh KV, Somasundaram G. Delineation of inheritance pattern of aleurone layer colour through chemical tests in rice. Rice 2017;10(1):1-0.
16. 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):304-11.
17. 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):177-9.
18. Sripunitha A, Sivasubramaniam K. Varietal characterization rice varieties based on chemical methods. Trends Biosci 2014;7(20):3139-46.
19. Singh C, Kumar SJ, KV S. Characterization and identification of rice germplasm accessions using chemical tests. Seed Research 2017;45(1):75-83.
20. Mathad RC, Channaveeraswamy AS, Kumar DV, Naik VR, Ashtaputre SA. Faster Chemical Methods to Determine Genetic Purity in Pigeon Pea (*Cajanus cajan* (L.) Mill sp.). Int. J Curr. Microbiol. App. Sci 2019;8(3):1378-92.
21. Nagendra MS, Selvaraju P, Jerlin R, Ganesamurthy K, Senthil N. Identification and characterization of popular rice (*Oryza sativa* L.) varieties through chemical tests. Journal of Phytology 2020;12:82-5.
22. Jaiswal JP, Agrawal RL. Varietal purity determination in rice: modification of the phenol test. Seed science and technology 1995;23(1):33-42.
23. Saharan VJ. Varietal Identification in Rice (*Oryza Sativa* L.). M.Sc. thesis, Haryana Agricultural University, Hisar, India 1991.
24. Cabaj J, Sołoducho J, Nowakowska-Oleksy A. Langmuir-Blodgett film based biosensor for estimation of phenol derivatives. Sensors and actuators B: Chemical 2010;143(2):508-15.
25. Fernandes KF, Lima CS, Lopes FM, Collins CH. Hydrogen peroxide detection system consisting of chemically immobilised peroxidase and spectrometer. Process Biochemistry 2005;40(11):3441-5.
26. Kumar SP, Prasad SR, Kumar M, Singh C, Sinha AK, Pathak A. Seed quality markers: a review. Res Rev 2016;3:13-7.
27. Steffens JC, Harel E, Hunt MD. Polyphenol oxidase. InGenetic engineering of plant secondary metabolism. Springer, Boston, MA 1994, 275-312.
28. Fuerst EP, Okubara PA, Anderson JV, Morris CF. Polyphenol oxidase as a biochemical seed defense mechanism. Frontiers in plant science 2014;5:689.
29. Takahashi N. Differentiation of ecotypes in *Oryza sativa* L. In Developments in crop science. Elsevier 1984;7:31-67.
30. Masuthi D, Vyakarnahal BS, Deshpande VK, Jagadeesha RC, Mukesh LC, Khaja Rubina S. Characterization of traditional aromatic rice cultivars by chemical markers. Int J Adv Res 2015;3(5):103-7.
31. Qian Q, He P, Zheng X, Chen Y, Zhu L. Genetic analysis of morphological index and its related taxonomic traits for classification of Indica/japonica rice. Science in China Series C: Life Sciences 2000;43(2):113-9.
32. Vanangamudi K, Palanisamy V, Natesan P. Variety determination in rice-phenol and potassium hydroxide tests. Seed science and technology 1988;16(2):465-70.
33. Gupta PK, Agrawal RL. Determination of varietal purity of paddy varieties by laboratory evaluation. Oryza 1988;25:310-4.