Biological Forum – An International Journal **15(8a): 300-306(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Characterization and Identification of Recent Rice varieties through Chemo Taxonomical Test

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(Received: 20 June 2023; Revised: 29 June 2023; Accepted: 21 July 2023; Published: 15 August 2023)
(Published by Research Trend)

ABSTRACT: The restricted genetic basis of the major rice varieties has resulted in a decrease in physical variation among them, necessitating the creation of efficient and reliable varietal identification tests, especially for the individuals who oversee and certify the quality of seeds. Ten rice varieties were tested for eleven different chemical tests such as phenol test, modified Phenol test, NaOH test, $FeSO_4$ test, KOH test, gelatinization temperature test, aroma test, GA_3 test, 2,4-D test, kinetin test, potassium iodide test. Based on how different genotypes' colour reactions affected the seed's ability to discriminate between the ten paddy varieties. Each of the eleven chemical tests separated the seed variety into two to six classes based on their responses. Even though no one test was able to characterize any specific variety, the eleven chemical tests together were able to distinguish each of the ten different varieties of rice. Thus, these chemical tests were straight forward, fast, and consistent and it might be used to distinguish between several rice cultivars.

Keywords: Chemical test, colour change, growth hormone, rice, varietal identification.

INTRODUCTION

In many parts of the world, especially many emerging nations in Asia, Africa, and Latin America, rice (*Oryza sativa* L.) is the main supper. Ever food shortages could occur soon as a result of declining agricultural resources and rising population. Therefore, since ancient times, consistent emphasis has been placed on rice crop improvement, leading to the introduction of numerous cultivars with better biotic and abiotic pressure tolerance or yield. Therefore, there is a broader selection of rice varieties available on the seed market for farmers to choose from, based on the agro-climatic conditions of the area, the variety's commercial value, agronomic practices, and the cropping style that should be applied to the particular genotype. So, it's crucial to characterise and identify a variety with the right traits in order to make it easier for the farmer to choose the variety of his choosing. In addition, variety characterization and identification satisfy numerous needs of various stakeholders, including those of breeders who want to gauge the diversity of their breeding stocks. Similar to how it helps testing authorities determine Prior to registering a variety under the PVP Act, it must be unique, it also helps certification authorities manage the following seed lot distribution and marketing. Most crucially, Farmers must select a suitable cultivar for commercial farming and characterising the crop is a must for helping with the decision-making process. Traditionally, morphological characteristics have been used to characterise and identify a variety. To do this, a variety's plants were cultivated, and morphological characteristics were observed in order to determine the variety's identity. However, even though morphological traits are the undeniably most reliable markers for varietal characterization and identification, they occasionally show limitations, such as the low degree of variation seen between varieties, which are brought on by the limited genetic diversity of the breeders' germplasm, effects of the environment on the traits expression, and the length of time required to see results. As a result, it is required to look for more descriptions that have strong environmental independence, repeatability, and discriminatory power. One such option is how different chemicals affect a

seed's coloration. Depending on their chemical or metabolite makeup, seeds that are exposed to specific chemicals grow in a particular colour. This colour is a variety-specific feature, and it has been successfully applied in a range of crops for varietal characterization and identification. For example, the FeSO4 test is utilized for foxtail millet, pearl millet, and sorghum; the modified phenol test is applied to wheat; the KOH test is suitable for rice, and so on. These uncomplicated chemical tests do not demand specialized scientific knowledge. When coupled with other straightforward traits like seed and seedling characteristics, their ability to differentiate between various types and effectively identify them is greatly enhanced. Consequently, both UPOV and PPVFRA have included the glume phenol reaction (referred to as the phenol test) as a DUS testing criterion for rice in their respective guidelines. The application of fundamental laboratory techniques like the KOH test in rice has been firmly established. Nonetheless, given the limited range of varieties examined in these published studies and the increasing number of varieties developed over time, it becomes imperative to assess the effectiveness of chemical tests for the characterization and identification of varieties. The present investigation aimed to determine how seeds from ten different commercial rice varieties would react to a range of chemical tests. These tests included the phenol test, modified phenol ($CuSO_4$) test, potassium hydroxide (KOH) test, sodium hydroxide (NaOH) test, among others. The primary objective was to assess whether these tests could effectively serve the purpose of identifying and characterizing the varieties.

MATERIAL AND METHODS

Seed source. This experimentation utilized seed samples from ten different rice varieties (ADT 53, ADT 54, ADT 55, ADT 57, TKM 15, MDU 6, CO 52, CO 55, VGD 1, TRY5), which were collected from various rice research stations in Tamil Nadu. These experiments were carried out at the Department of Seed Science & Technology, Agricultural College and Research Institute, Madurai. The rice varieties underwent a series of chemical tests, including phenol, sodium hydroxide, ferrous sulphate, potassium hydroxide, modified phenol, gelatinization, aroma, GA_3, 2,4-D, KI, and kinetin tests.

Phenol test. In the phenol assessment, three sets of twenty-five seeds from each type were soaked in distilled water for a day at 25°C. Afterward, the seeds were placed on petri plates lined with filter paper and exposed to a 1% phenol solution dampened with 10 ml, and were left at ambient temperature (28°C) for a day. The samples were then graded according to color transformation, categorized as no color alteration, light brown, or brown (Walls, 1965).

Modified phenol test. In the adapted phenol examination, three repetitions of twenty-five seeds for each variation were utilized. The procedure closely mirrored that of the typical phenol test, with the exception that instead of soaking the seeds in distilled water, they were submerged in a 0.5 percent $CuSO_4$ solution (15 ml) for a duration of 24 hours. Following this, the seeds were positioned atop damp filter paper along with 10 ml of a 1% phenol solution. The assessment involved discerning and categorizing the seed coat's colour response into distinct classes like brown, light brown, and no change in colour, as detailed by Vishwanath *et al.* (2013).

Ferrous sulphate test ($FeSO_4$). For the FeSO4 treatment, three sets of twenty-five seeds each were submerged in a 1.5 percent $FeSO_4$ solution for 4 hours at room temperature. Subsequently, the seeds were extracted from the solution and excess moisture was eliminated by utilizing blotting paper prior to assessment. The seeds were scrutinized to identify colour changes and were classified into categories such as Dark Grey Streaks (DGSt), Brown Streaks (BSt), and Brown Spots (BSp), following the classification system outlined by Gupta and Agrawal (1988).

Sodium hydroxide test (NaOH test). For the NaOH assessment, three sets of twenty-five seeds each per variety were employed. The alteration in solution color was observed subsequent to immersing the seeds in 15 ml of a 5% NaOH solution and allowing them to incubate at room temperature for a duration of 5 hours. The genotypes were categorized into three groups, distinguished by the intensity of the colour response: no change in colour, light yellow, and deep yellow hues, as outlined by Chakrabarty and Agarwal (1989).

Potassium hydroxide test (KOH test). For the KOH examination, three sets of twenty-five seeds each from every variation were submerged in 15 ml of a 5% KOH solution for a period of 5 hours. Following this, the cultivars were sorted into categories based on the intensity of the colour transformation, ranging from pale yellow to dark yellow, or no change in colour, in accordance with the classification system presented by Masuthi *et al*. (2015).

Gibberellic acid test (GA_3 test). The experiment began by subjecting rice seeds to surface sterilization using distilled water. These seeds were then placed onto a double-layered blotter that had been moistened with a 25ppm GA_3 solution, following the procedure outlined in ISTA (1996). The incubation took place at a temperature range of 25±10°C. As a control, another set of seeds was placed on a water-soaked blotter without the GA3 solution. After 14 days of incubation, the germinated seeds were observed, and the length of ten randomly selected seedlings was measured to determine the percentage increase in seedling length compared to the control. The percentage increase in coleoptile length over the control was calculated using the provided formula

$$\% \text{ increase in coleoptile length over control} = \frac{\text{Length of } GA_3 \text{ treated coleoptile} - \text{length of coleoptile with control}}{\text{Length of coleoptile with control}} \times 100$$

The seeds were categorized based on the percentage increase in coleoptile length compared to the control in the following manner:

a) Very low response: < 10 per cent increase
b) Low response: 10-30 per cent increase
c) Moderate response:> 30 percent increase.

2,4-D auxin test (2,4-D test)

In each variety, a set of twenty-five seeds underwent a 24-hour soaking period in a 5ppm solution of 2,4-Dichlorophenoxyacetic acid. Subsequently, these treated seeds were positioned on moist germination paper in two separate replications, alongside two replications of untreated control seeds. The setup was then rolled up. The germination papers, along with the seed setups, were transferred to a germination room that was maintained according to the specifications in the ISTA procedure (1996) – a temperature of 25±10°C and a relative humidity of 90±3%. After a 14-day incubation period, the shoot lengths of ten randomly selected seedlings were measured. Using the obtained measurements, the percentage decrease in seedling length was calculated using the method provided below

$$\% \text{ decrease in seedling length} = \frac{\text{Reduction in shoot length of } 2,4\text{-D soaked seeds over control}}{\text{Seedling length of control}} \times 100$$

The categorization of the varieties was determined by the percentage change in seedling length, either in terms of increase or decrease, as follows:

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

Kinetin soak test. In each variety, twenty-five seeds underwent a 24-hour soaking in a solution of 25 ppm kinetin. Subsequently, these treated seeds were positioned on damp germination paper in two separate replications, alongside an untreated control group, and then carefully rolled to prevent spillage. The assemblies of seeds were placed in the germination room, which was maintained at a temperature of 25±2°C and a relative humidity of 90±3%, following the procedure outlined in ISTA (1996). After a 14-day incubation period, the shoot lengths of ten randomly selected seedlings were measured. Using these measurements, the percentage increase in seedling length was calculated using the provided method

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$$\% \text{ of increase /decrease in seedling length} = \frac{\text{Increase/decrease in seedling length of kinetin soaked seeds over control}}{\text{Seedling length of control}} \times 100$$

The categorization of the varieties was determined by the extent of increase or decrease in seedling length, as follows:

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

Gelatinization temperature test. Ten decorticated seeds from each variety were subjected to three separate replications. These seeds were soaked in petri plates containing 10 ml of a 1.7% solution of Potassium Hydroxide (KOH). The petri dishes were then left undisturbed at a temperature of 30°C for a duration of 24 hours, following the procedure described by Little (1958). The gelatinization temperature test, as recommended by the Protection of Plant Varieties and Farmers' Rights Authority, was performed. The kernels' responses to the alkali treatment were assessed and recorded using a seven-point scale, as specified.

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. In a test tube, five grams of decorticated seeds were placed along with 15 ml of double-distilled water and left to soak for a duration of 10 minutes. Following this, the test tubes were subjected to heating at 80°C for 15 minutes. The resulting contents were subsequently transferred to petri dishes and allowed to cool in a refrigerator for 20 minutes. Based on the aroma detected, the samples were categorized into three groups: strongly scented, mildly scented, and non-scented, following the method outlined by Nagendra *et al.* (2020).

KI test. The dehusked seeds were sliced at their center to reveal the endosperm. This exposed endosperm was then submerged in a mild KI solution (0.2% Iodine + 2% KI) for a duration of five minutes and closely examined for any color transformation. In this process, the glutinous endosperm would take on a brownish hue, while the non-glutinous endosperm would appear bluish-brown, as per the methodology described by Masuthi *et al.* (2015).

RESULTS AND DISCUSSION

In comparison to traditional methods like the grow out test (GOT), which require more space and time to cultivate a crop, chemical tests are quick, inexpensive, and time-effective. Morphological observations should be made throughout the crop growth cycle from planting to harvest (Vishwanath *et al*., 2013). The results of a chemical test based on an enzymatic or chemical reaction between a chemical solution and a seed coat can be seen within a few hours in Phenol, KOH, $FeSO_4$ etc. Under such conditions a number of tests in combination can resolve themselves into a key for identification of varieties. Keeping this in view, a critical evaluation for various techniques has been employed for identification of varieties.

Phenol test: The phenol color reaction revealed that the ten rice genotypes under investigation could be categorized into three distinct color groups. Among these, four varieties, namely ADT-54, ADT-57, TKM-

15, and CO-52, exhibited a brown color reaction. In contrast, ADT-53, MDU-6, and ADT-55 displayed a light brown color, while the remaining three varieties, VGD-1, TRY-5, and CO-55, did not exhibit any color change, as indicated in Table 1 and Fig. 1. Sivasubramanian and Ramakrishnan (1978) have previously established that the Phenol color test, which involves the oxidation of phenol into a dark melanin color catalyzed by the tyrosinase enzyme, is a straightforward, rapid, and accurate method for grouping rice varieties. The phenol test was chosen as the key diagnostic characteristic for differentiating paddy types due to the strong heritability and stability. A thorough examination of the findings reveals that, despite being a quick and reliable test, it is insufficient on its own to identify all the kinds since it divides them into various groups according on how they react to color. As a result, numerous additional chemical tests are required to differentiate between all the kinds.

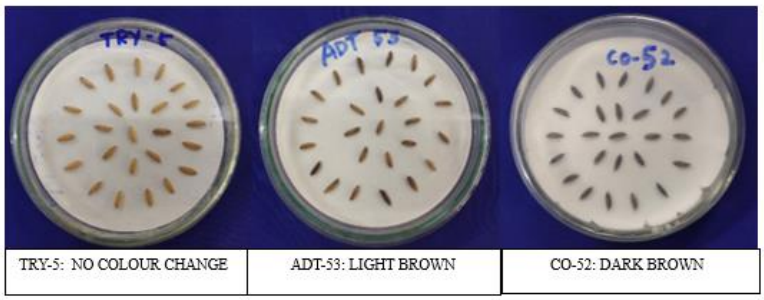

Fig. 1. Phenol test.

Modified phenol test. The modified phenol test independently categorizes all ten varieties into three groups, akin to the phenol test. Among the ten varieties, seven—namely ADT-53, ADT-54, ADT-57, TKM-15, MDU-6, CO-52, and CO-55—displayed a light brown color reaction. In contrast, the remaining three varieties, namely VGD-1, TRY-5, and ADT-55, did not exhibit any color change, as outlined in Table 1. The color reaction in several types is significantly improved by adding Cu+ ions to the modified test. However, the combination of the phenol test and the modified phenol test, which is an easy, affordable, and speedy way to discern the different rice varieties, may be a good option to categorize and identify the types. According to Banerjee and Chandra (1997), modifying the phenol test by introducing copper and sodium ions for a period of six hours enables the identification of different wheat cultivars.

Table 1: Impact of chemical tests on variations in seed color.

Sr. No.	Rice variety	Phenol test	Modified phenol test	$FeSO_4$ test	NaOH test	KOH test	KI test
1.	ADT-53	Light brown	Light brown	Brown streak	Dark yellow colour	Dark yellow colour	Brown
2.	ADT-54	Brown	Light brown	Brown streak	Pale yellow	Pale yellow	Brown
3.	ADT-55	Light brown	No colour change	Brown streak	Pale yellow	Pale yellow	Brown
4.	ADT-57	Brown	Light brown	Brown streak	Pale yellow	Pale yellow	Brown
5.	TKM-15	Brown	Light brown	Brown streak	Pale yellow	Pale yellow	Brown
6.	MDU-6	Light brown	Light brown	Brown streak	Pale yellow	Pale yellow	Brown
7.	CO-52	Brown	Light brown	Brown streak	Pale yellow	Pale yellow	Brown
8.	CO-55	No colour change	Light brown	Brown streak	Pale yellow	Pale yellow	Brown
9.	TRY-5	No colour change	No colour change	Brown streak	Pale yellow	Pale yellow	Brown
10.	VGD-1	No colour change	No colour change	Brown streak	Pale yellow	Pale yellow	Brown

Ferrous sulphate test ($FeSO_4$). In the current investigation, the ferrous sulphate test exhibited a positive response, yet no discernible variations were noted among the different varieties. All the tested varieties displayed a uniform brown-coloured streak at the seed's core, as outlined in Table 1 and Fig. 2. Due to its user-friendly nature and straightforward application, the ferrous sulphate test proves valuable in distinguishing between genotypes based on seed secondary metabolites. Gupta and Agrawal (1988) highlighted that the color reaction on rice husks served as an effective diagnostic feature for categorizing paddy

varieties, achieved through immersion in a 1.5 percent ferrous sulphate solution for four hours at room temperature, resulting in classifications as brown spots, dark grey spots, and brown streaks. Conversely, Reddy *et al.* (2008) indicated that the ferrous sulphate reaction did not manifest cotton cultivar variability.

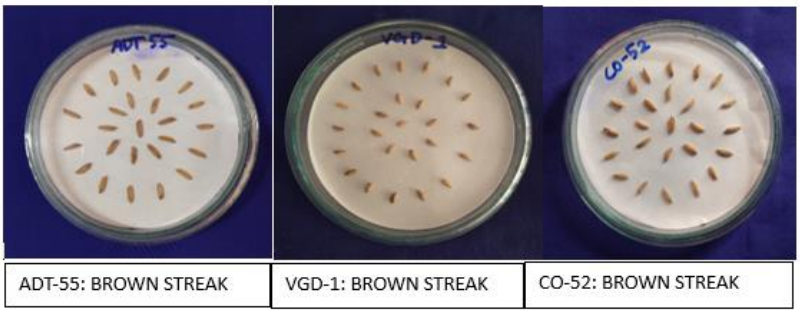

Fig. 2. Ferrous sulphate test.

Sodium hydroxide test (NaOH). Various rice varieties exhibited diverse reactions to the sodium hydroxide test. Depending on the color change in the poured-off solution, these varieties were categorized into three groups: those with a dark yellow hue, those with a pale yellow, and those that exhibited no color alteration. The differing responses of the NaOH and KOH test solutions can be attributed to variations in the chemical composition and genetic characteristics of the cultivars. The presence of secondary metabolites and inherent chemical distinctions within the seeds of the ten tested types likely contributed to the observed variations in color.

Regarding the sodium hydroxide test, all the cultivars demonstrated a positive response to the sodium hydroxide solution. Out of the ten rice varieties, the soak solution of nine cultivars turned pale yellow, while ADT-53 alone displayed a transformation into a dark yellow color, as detailed in Table 1. Vanderburg and Vanzwol (1991) asserted that the reactivity of sesame seeds to secondary metabolites was responsible for the color change reaction observed when exposed to a sodium hydroxide solution. To distinguish between rice cultivars, the leachates from the seeds react with the alkali to produce a color of varying intensity (Muthu *et al.*, 2022).

Potassium hydroxide test (KOH). The exudates from the seeds interact with the alkali, resulting in a spectrum of colours that can be utilized for distinguishing various rice cultivars. In the current investigation, all ten rice varieties exhibited positive responses to the potassium hydroxide test. Based on the progression of color in the drained solution, the varieties were categorized into three groups: those with a dark yellow tint, those with a pale yellow, and those that displayed no color alteration. Among the ten rice varieties, the seed soak solution of nine cultivars displayed a transformation into a pale yellow, while only ADT-53 turned a shade of dark yellow, as detailed in Table 1. Comparable observations were also made by Raut *et al.* (2019); Mathad *et al.* (2019).

Coleoptile response to GA3 test. GA3 serves as a growth hormone, and the way seedlings react to this hormone hinges on its intrinsic presence. Varieties were categorized into low, very low, and moderately responsive groups based on their reactions. Within the set of ten varieties, ADT-54 was categorized as very low response and ADT-53, ADT-57, TKM-15, MDU-6, CO-52, CO-55, TRY-5, VGD-1 were categorized as low response, while ADT-55 possessed moderate response to GA3 (Table 2). The increased shoot length of GA3-treated seeds may be the result of the cell's elongation, which would have caused the coleoptile and shoot to grow longer (Bachelard, 1968; Mohan Ram and Mehta 1978).

Table 2: Influence of chemical tests on diversity in seed color and seedling growth.

Sr. No.	Rice variety	GA_3 test	2,4-D test	Kinetin soak test	Gelatinization temperature test	Aroma test
1.	ADT-53	Low	Very low	Moderate	Kernel swollen	Non scented
2.	ADT-54	Very low	Moderate	Moderate	Kernel swollen	Non scented
3.	ADT-55	Moderate	Moderate	Moderate	Kernel swollen	Non scented
4.	ADT-57	Low	Moderate	Moderate	Kernel swollen	Non scented
5.	TKM-15	Low	Low	Low	Kernel swollen	Non scented
6.	MDU-6	Low	Very low	Moderate	Kernel swollen	Non scented
7.	CO-52	Low	Low	Moderate	Kernel swollen	Non scented
8.	CO-55	Low	Very low	Low	Kernel swollen	Non scented
9.	TRY-5	Low	Low	Moderate	Kernel swollen	Non scented
10.	VGD-1	Low	Low	Moderate	Kernel swollen	Non scented

Coleoptile response to 2,4-D. 2,4-D functions as a growth hormone, and the seedling's reaction to this hormone relies on its naturally occurring presence within the plant. The cultivars were categorized into groups of low, very low, and moderate responsiveness based on their respective reactions. Out of ten varieties ADT-53, MDU-6, CO-55 was categorized as very low response, while TRY-5, VGD-1, TKM-15, CO-52 possessed low response to2,4-D. ADT-54, ADT-55, ADT-57 were grouped into moderate response (Table 2). The herbicidal action of 2,4-D, which has the capacity to inhibit the manufacture of phospholipid and enhance the creation of free radicals, can be used to explain why the shoot length of 2,4-D-treated seeds reduced (Hume and Shirriff 1989; Ozkul *et al.,* 2016).

Kinetin soak test. Based on the seedling growth responses of paddy varieties to kinetin the varieties were grouped into low, very low and moderate responsive. Varieties viz., TKM-15, CO-55 exhibited low percentage increase in the seedling length whereas, ADT-53, ADT-54, ADT-55, ADT-57, MDU-6, TRY-5, CO-52, VGD-1 exhibited moderate percentage decrease in the length (Table 2). The difference in shoot length of cytokinin-treated seeds may be caused by a delay in the stomata's closure at higher concentrations, and the growth inhibition may be caused by the metabolic activity's failure to be triggered by faulty absorption at lower concentrations (Wang *et al*., 2015).

Gelatinization temperature test. All the varieties exhibited low alkali spreading value of 2 (Kernel swollen) which correspond to high gelatinization temperature and there was no significant differentiation among the tested paddy varieties for gelatinization temperature test (Table 2). All the ten tested varieties had high gelatinization temperature. Regardless of the amount of protein in the rice grain, the gelatinization temperature is strongly associated with the amylose level (Saied *et al*., 1979). Globally, medium to high alkali spreading values and gelatinization temperatures are recommended because they produce the best cooking quality (Pushpa *et al*., 2019).

Aroma test. There was uniformity among the varieties of the aroma test, with all of them being non-scented types as indicated in Table 2. These outcomes align with the findings of Geetha *et al.* (2011) in their study on three different rice varieties (TRY 3).

KI test. The genotypes were classified into two groups, namely brown and bluish-brown responses to the KI test, based on the alteration in the solution's colour. All the ten varieties exhibited brown colour to the KI test (Table 1). Similar results were also noted by Masuthi *et al*. (2015).

CONCLUSIONS

The findings concluded that individual chemical tests have limited utility and these tests may distinguish any number of varieties when performed in a complimentary sequence or in combination. In order to identify the rice crop varieties, these straightforward, quick, and reliable tests are of great utility. The study also showed that, these tests might be used to accurately determine the purity of the rice variety for regular testing.

Acknowledgement. I express my sincere gratitude to Dr. V. Alex Albert, Dr. K. Sujatha, Dr. K. Thangaraj, Dr. B. R. Renuka and all other faculty members of Department of Seed Science and Technology, Agricultural College and Research Institute, Madurai for the support and guidance to carry out this research study.

Conflict of Interest. None.

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How to cite this article: N. Aarthi, V. Alex Albert, K. Sujatha, K. Thangaraj and B.R. Renuka (2023). Characterization and Identification of Recent Rice varieties through Chemo Taxonomical Test. *Biological Forum – An International Journal*, *15*(8a): 300-306.