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Quantification of NaOH and KOH Tests for Varietal Identification in Traditional Rice Varieties - A New Approach

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ABSTRACT: Traditional varieties in rice are gaining momentum because of its nutritive value. The seed production and use of such varieties in breeding programme are the trends now. Therefore, the genetic purity and its identification are necessary. Hence, an experiment to explore the possibility of quantifying the chemical tests *viz*., NaOH and KOH for varietal identification and genetic purity assessment in traditional rice varieties was conducted in the Department of Seed Science and Technology, Seed Centre, Tamil Nadu Agricultural University, Coimbatore, India. The results indicated that the traditional rice varieties have large variations in grain colour and thus, it is considered an important trait for varietal characterization and identification. In parallel, the chemical tests *viz*., NaOH and KOH also showed better differences among the varieties and these tests can very well used for the identification of red grain and white grain varieties. In addition, the measurement of colour absorbance of the NaOH and KOH tests through UV-Visual Spectrophotometer at 430 nm and colour intensity by Tintometer can be used for further differentiation of the varieties.

Keywords: Rice, Traditional varieties, Landraces, Varietal identification, Chemical tests, NaOH test, KOH test

Rice is the most consumed and common food of everyday diet in many parts of the world. It is a stable food for more than 3.5 billion people living in Asia, Latin America and parts of Africa. It is the belief of scientists that people first domesticated rice in India or Southeast Asia. The largest rice producers in the world are China, India and Indonesia. Rice is cultivated over an area of 165 million hectares in the world and 43.86 million hectares in India and the country produces 122.304 tonnes of rice [1]. Now-a-days, traditional rice varieties are gaining importance and it is cultivated by a large number of farmers. These varieties play a major role in history of crop improvement and production and they offer various health benefits [2]. However, these varieties are severely threatened by genetic extinction due to the increase in homozygous modern genetic cultivars. This causes a concern among the breeders, farmers and conservationists [3].

The genuineness of the variety is one of the most important characteristics of the quality of the seed. Generally, the landraces show wider variation in its morphological and biochemical traits. Therefore, identification and characterization of these varieties are essential for maintaining the genetic purity. In which, the seed and grain traits are considered as essential for identification of the varieties. In this regard, the seeds in some varieties may have yellow testa but grains are coloured vice versa, the testa may be coloured with white grains. In this case, some white grain seeds may be present in the coloured rice which is to be of off-types. Therefore, such white coloured grains are need to be identified for assessing the genetic purity. Thus, the chemical tests are important to identify those kind of seeds. Further, the chemical methods were used for varietal identification [4, 5, 6, 7] which proved that the tests are rapid, distinct and reproducible. Among which, the tests like NaOH and KOH were used for the differentiation of white rice and red rice varieties. However, the colour change in the solution of white rice and red rice varieties is very narrow in the chemicals tests and thus, it is difficult to differentiate them. Hence, an experiment was conducted to measure the colour spectrum during the chemical tests conducted in traditional rice varieties.

MATERIALS AND METHODS

The seeds of 65 traditional rice varieties were collected from the farmer’s holdings of various parts of Tamil Nadu *viz*., Cuddalore, Thanjavur, Nagapattinam, Madurai, Tirunelveli, Thiruvarur and Kanyakumari districts. Multiplication of seeds of the collected varieties was carried out in the Wetlands farm, Tamil Nadu Agricultural University, Coimbatore. Simultaneously, purification of the varieties was carried out by removing the off-type plants based on plant, flower and seed morphological traits. The seeds were harvested and cleaned thoroughly. Then, the varietal characterization was carried out in the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore. The hulled grains of all the varieties were observed under natural light conditions for colour by using Royal Horticultural Society (RHS) colour chart and were classified into different categories. Then, the chemical tests *viz*., NaOH and KOH were carried out for the genetic purity assessment.

Sodium hydroxide (NaOH) test

Three replications containing 25 seeds each were soaked in 10 ml of 5 percent 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 [8].

Potassium hydroxide (KOH) test

Twenty-five seeds of each landrace in three replications were soaked in test tubes containing 10 ml of 5 percent 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 [9].

Measurement of colour absorbance

Then, the solutions of NaOH and KOH tests conducted were measured for its colour absorbance by using UV-Visible Spectrophotometer (Model: SP- 3000) [10]. Generally, the colour of the solutions was ranged from light yellow to red. Therefore, the absorbance for the solution was measured using multiple wavelengths from 430 to 520 nm for standardizing a particular wavelength. Based on the absorbance recorded at multiple wavelengths, the maximum absorbance was noticed at 430 nm and it was fixed as standard for measurement. Therefore, the absorbance of the NaOH and KOH solutions soaked with the varieties was measured at 430 nm and expressed in OD.

Measurement of colour intensity

Also, the solutions obtained from NaOH and KOH tests were measured for its colour intensity by using tintometer (Model F (BS 684). The tintometer expresses colour in terms of Lovibond® Red, Yellow, Blue and Neutral. For measuring the colour intensity, the samples were compared against the blank (white colour) in which all the attributes measured *viz*., l, a, b and c with 0. Then, the colour of the samples was measured by placing the lens above the sample in a minimum light area. After measurement, the “E or DE* (colour difference assessment from a recognized standard) was calculated by using the following formula,

$$DE^*ab = [(DL)^2 + (Da)^2 + (Db)^2]^{1/2}$$

$$DE^*ab = [DE^*)^2 + (DC^*)^2 + (DH^*)^2]^{1/2}$$

Where,

DL* = modification in lightness/darkness value

Da* = modification on red/green axis

Db* = modification on yellow/blue axis

DC* = modification in chroma

DH* = modification in hue

DE* = total colour difference value

Cluster analysis

Then, the colour responses in chemical tests were transformed to binary characters such as 0, 1, 2, 3, and 4 and analyzed further [11]. The two sets of data which defines the association between traditional rice varieties *viz,* qualitative and quantitative traits observed were analyzed using cluster analysis. In which, the data were analyzed using Agglomerative Hierarchical Clustering (AHC) which was performed by means of Ward’s method. The resulting similarity matrix was subjected to cluster analysis which measures using Euclidean distance.

A dendrogram depicting the number of clusters formed was generated by Minitab software as a result of data analysis which was used for further interpretation [12].

RESULTS AND DISCUSSION

Generally, the traditional rice varieties have wider variations in grain colour and it is considered as one of the important traits for varietal identification. In this regard, the hulled grains were classified based into white, light brown, variegated brown, dark brown, light red, red, variegated purple, purple and dark purple. In the present study, the varieties *viz*., Idly, Kallundrikar, Kallurundaikar, Kandasaali, Karukka, Karuppu kavuni, Kattuyanam, Kothamalli samba, Kuzhiyadichan, Mottakur, Paalthondi, Rangoon samba and Sorna have recorded with dark brown grains. While, variety Muthuvellai had light brown grain and Vellaigundu samba with dark purple grain whereas, the varieties such as Arubadhamkuruvai, Chinnar, Chithiraikar, Karunkuruvai, Kattanur, Kullankar, Kuruvaikalanjium, Mapillai samba, Mysore malli, Nootripathu, Norungan, Poongar, Poovan samba, Samba mosanam, Vaasanai seeraga samba, Vadakathi samba and Varakkal had red grains (Table 1). Similar results of variation in grain colour were obtained earlier in rice [13, 14, 15,16].

The colour reaction with NaOH solution classifies the traditional rice varieties into light yellow, yellow, pale red and red with 19, 15, 7 and 24 varieties in each category. The varieties such as Arasamba, Atturkichili samba, Katta samba, Korangu samba, Kudhiraivaali samba, Maranel, Muthuvellai, Nalannamak, Palkachaka, Ponmani samba, Purple puttu, Seeraga samba, Sornamasuri, Thengaipoo samba, Thooyamalli, Thulasivaasanai, Uppumolagi, Varigarudan samba and Vellai kavuni changed the NaOH solution into light yellow and the varieties like Illupaipoo samba, Kaalabadh, Kalanamak, Kallukar, Manjalponni,

Table 1. Response of the seeds of traditional rice varieties to NaOH and KOH tests

S. No.	Variety	Seed colour	Grain colour	NaOH test	KOH test	Absorbance (OD value)		Colour intensity (ΔE)	
						NaOH test	KOH test	NaOH test	KOH test
1.	Anaikomban	Straw (161A)	White (158A)	Yellow	Light yellow	1.687	1.406	95.1	95.9
2.	Arasamba	Brown furrows (N167A)	White (159C)	Light yellow	Light yellow	0.502	0.548	98.7	93.8
3.	Arubadhamkuruvai	Brown furrows (N167A)	Red (175C)	Red	Pale red	2.422	1.522	96.0	94.7
4.	Atturkichili samba	Straw (161A)	White (158A)	Light yellow	Light yellow	0.910	0.966	96.1	95.5
5.	Chinnar	Brown furrows (N165B)	Red (175C)	Red	Red	2.443	2.857	95.8	97.0
6.	Chithiraikar	Brown furrows (N167A)	Red (175C)	Red	Red	2.214	2.700	97.8	97.0
7.	Idly	Black (202A)	Dark brown (178B)	Pale red	Red	2.466	2.348	95.4	96.5
8.	Illupaipoo samba	Black (202A)	White (159A)	Yellow	Yellow	1.923	1.705	95.2	96.3
9.	Kaalabadh	Black (202A)	White (185A)	Yellow	Yellow	1.001	1.290	95.7	94.4
10.	Kalanamak	Straw (161A)	White (159C)	Yellow	Yellow	1.910	1.920	94.0	95.9
11.	Kallukar	Brown furrows (165A)	White (158A)	Yellow	Light yellow	1.773	1.206	91.0	95.3
12.	Kallundrikar	Brown furrows (163B)	Dark brown (175B)	Red	Red	2.238	2.408	96.2	96.6
13.	Kallurundaikar	Brown furrows (165A)	Dark brown (175B)	Red	Red	2.125	2.746	96.9	97.9
14.	Kandasaali	Brown furrows (164A)	Dark brown (175B)	Red	Red	2.640	2.528	97.0	98.1
15.	Karukka	Gold and gold furrows on straw background (162A)	Dark brown (183B)	Red	Pale red	2.588	1.659	96.6	97.4
16.	Karunkuruvai	Black (202A)	Red (175C)	Red	Red	2.343	2.330	96.7	96.5
17.	Karuppu kavuni	Black (202A)	Dark brown (175A)	Red	Red	2.564	2.889	95.9	97.0

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S. No.	Variety	Seed colour	Grain colour	NaOH test	KOH test	Absorbance (OD value)		Colour intensity (ΔE)	
						NaOH test	KOH test	NaOH test	KOH test
18.	Katta samba	Straw (161A)	White (158A)	Light yellow	Light yellow	1.019	0.543	90.3	94.3
19.	Kattanur	Gold and gold furrows on straw background (22A)	Red (175C)	Pale red	Pale red	1.964	1.972	97.7	96.4
20.	Kattuyanam	Brown furrows (167A)	Dark brown (178A)	Red	Red	2.226	2.800	96.0	96.2
21.	Korangu samba	Brown furrows (N167A)	White (159A)	Light yellow	Light yellow	0.863	0.135	92.9	95.3
22.	Kothamalli samba	Gold and gold furrows on straw background (162A)	Dark brown (175A)	Red	Red	2.499	2.287	96.2	96.1
23.	Kudhiraivaali samba	Straw (161B)	White (158C)	Light yellow	Light yellow	1.040	1.183	94.7	96.5
24.	Kullankar	Black (N200A)	Red (175C)	Red	Red	2.976	2.833	96.7	96.6
25.	Kuruvaikalanjium	Brown furrows (163A)	Red (178A)	Red	Red	2.362	2.790	97.0	96.9
26.	Kuzhiyadichan	Brown furrows (165B)	Dark brown (183A)	Pale red	Red	1.912	2.859	96.9	97.0
27.	Manjalponni	Straw (161B)	White (156C)	Yellow	Yellow	1.067	1.769	95.0	95.1
28.	Mapillai samba	Brown furrows (N167A)	Red (183A)	Red	Red	2.646	2.364	98.8	97.8
29.	Maranel	Straw (161A)	White (159A)	Light yellow	Light yellow	0.692	0.645	95.3	95.5
30.	Mattaikar	Straw (161A)	White (158C)	Yellow	Yellow	1.163	1.817	98.0	94.4
31.	Milagu samba	Straw (161A)	White (159A)	Yellow	Light yellow	1.907	0.783	95.4	96.6
32.	Mottakur	Brown furrows (163A)	Dark brown (183B)	Red	Red	2.993	2.783	93.2	96.7
33.	Muthuvellai	Brown (tawny) (167A)	Light brown (174A)	Light yellow	Light yellow	0.904	1.526	97.6	95.1
34.	Mysore malli	Brown furrows (163A)	Red (175C)	Pale red	Pale red	1.126	1.704	96.1	97.1
35.	Naatu basmati	Brown (tawny) (163B)	White (159A)	Yellow	Light yellow	1.723	1.105	96.2	95.8
36.	Nalan namak	Straw (161A)	White (159A)	Light yellow	Light yellow	0.900	0.693	94.9	96.2
37.	Navara	Straw (161A)	White (156A)	Yellow	Yellow	1.096	1.739	96.4	94.6
38.	Nootripathu	Brown spots (162B)	Red (176A)	Red	Red	2.548	2.548	95.8	95.9
39.	Norungan	Brown furrows (N167A)	Red (176A)	Red	Red	2.623	2.947	96.1	98.0
40.	Paalthondi	Straw (161A)	Dark brown (175B)	Red	Red	2.708	2.505	99.7	95.9

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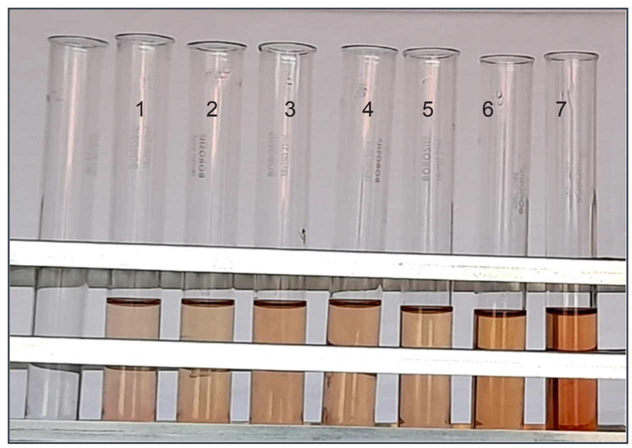
S. No.	Variety	Seed colour	Grain colour	NaOH test	KOH test	Absorbance (OD value)		Colour intensity (ΔE)	
						NaOH test	KOH test	NaOH test	KOH test
41.	Palkachaka	Straw (161B)	White (158A)	Light yellow	Light yellow	0.724	0.659	96.0	95.8
42.	Ponmani samba	Brown furrows (164B)	White (158A)	Light yellow	Light yellow	1.174	0.851	94.8	95.6
43.	Poongar	Brown furrows (N167A)	Red (175C)	Red	Pale red	2.490	1.660	96.6	95.0
44.	Poovan samba	Brown furrows (N167A)	Red (175C)	Red	Pale red	2.397	1.415	99.2	93.3
45.	Purple puttu	Straw (161B)	White (159A)	Light yellow	Light yellow	0.818	0.472	96.0	96.1
46.	Rajamannar	Straw (161B)	White (155B)	Yellow	Yellow	1.339	1.222	89.2	96.2
47.	Rangoon samba	Straw (161A)	Dark brown (174A)	Red	Red	2.938	2.014	96.7	95.3
48.	Salem sanna	Brown (tawny) (163B)	White (158B)	Yellow	Yellow	1.962	1.644	94.9	96.9
49.	Samba mosanam	Straw (161A)	Red (175C)	Pale red	Red	1.716	2.297	94.0	97.3
50.	Seeraga samba	Gold and gold furrows on straw background (162A)	White (158A)	Light yellow	Light yellow	0.230	0.463	93.9	96.0
51.	Sembuli samba	Brown (tawny) (163B)	White (156B)	Yellow	Light yellow	1.550	1.775	98.0	94.5
52.	Sivan samba	Straw (161A)	White (156A)	Yellow	Light yellow	0.895	0.405	95.8	88.2
53.	Sorna	Straw (161B)	Dark brown (175B)	Pale red	Pale red	1.847	1.737	96.8	95.7
54.	Sorna masuri	Straw (161A)	White (156A)	Light yellow	Light yellow	0.617	0.506	94.2	96.7
55.	Thanga samba	Straw (161A)	White (156B)	Yellow	Light yellow	0.587	0.593	95.0	95.5
56.	Thengaipoo samba	Straw (161A)	White (161C)	Light yellow	Light yellow	0.606	0.552	95.2	91.2
57.	Thooyamalli	Straw (161A)	White (156A)	Light yellow	Light yellow	0.638	0.616	94.2	95.1
58.	Thulasi vasanai	Gold and gold furrows on straw background (162A)	White (159C)	Light yellow	Light yellow	0.563	0.675	94.9	95.3
59.	Uppumolagi	Straw (161 C)	White (159C)	Light yellow	Light yellow	0.682	1.089	95.0	95.9
60.	Vaasanaiseeraga samba	Brown furrows (164 B)	Red (175C)	Red	Pale red	2.855	1.291	99.2	97.0
61.	Vadakathi samba	Straw (161B)	Red (175C)	Pale red	Pale red	1.305	0.979	94.9	94.2
62.	Varakkal	Brown furrows (165A)	Red (175C)	Red	Pale red	2.711	1.942	96.9	97.7
63.	Varigarudan samba	Straw (161B)	White (158A)	Light yellow	Yellow	0.801	1.433	91.6	94.7
64.	Vellaigundu samba	Black (202A)	Dark purple (202A)	Red	Red	2.245	2.652	93.6	95.2
65.	Vellai kavuni	Straw (161A)	White (155A)	Light yellow	Light yellow	0.049	0.030	95.8	95.0

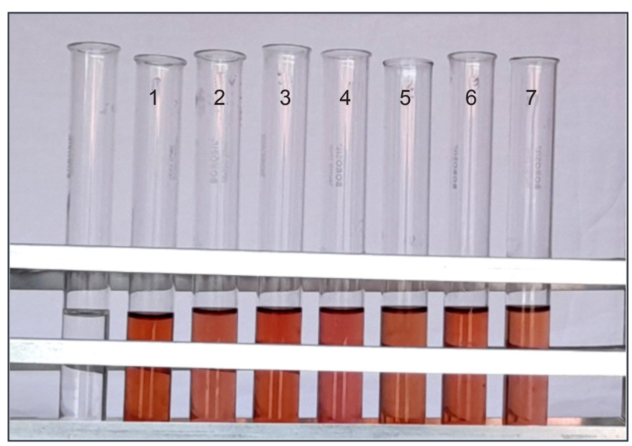
Mattaikar, Milagu samba, Naatu basmati, Navara, Salem sanna, Sembuli samba, Sivan samba, Thanga samba and Vadakathisamba changed the solution to yellow (Table 1). The remaining varieties *viz*., Chinnar, Chithiraikar, Idly, Kallundrikar, Kallurundaikar, Kandasaali, Karunkuruvai, Karuppu kavuni, Kattuyanam, Kothamalli samba, Kullankar, Kuruvaikalanjium, Kuzhiyadichan, Mapillai samba, Mottakur, Nootripathu, Norungan, Paalthondi, Rangoon samba, Samba mosanam and Vellaigundu samba grouped under red grain category changed the solution to pale red and red colour (Fig. 1). This is because of the presence of red pigment on the grain which influenced the colour change in the solution. The results are in accordance with the findings in rice [17-23], sorghum [24], greengram, cotton, sunflower [25], Indian mustard [26], tomato [27] and chilli [4]. Vishwanath *et al*. [27] opined that the variation in colour change during NaOH test was because of the difference in the seed constituents. The seed chemical composition was basically determined by the genetic makeup of the cultivars and hence, the colour variation was observed [26]. Shao *et al*. [28] observed that the hydroxy fatty acid component of the outermost cuticle was released during NaOH treatment in soybean and holes were produced in it, allowing the seeds to become permeable. Thus, it may be concluded that NaOH and KOH causes holes in the seeds through which the melanin of the grains gets extracted and are visible in the NaOH solution and is one of the common methods to extract natural melanin [29].

In the same way, the rice varieties were grouped into light yellow, yellow, pale red and red based on the colour change of the KOH solution. The red kernelled varieties like Chinnar, Chithiraikar, Idly, Kallundrikar, Kallurundaikar, Kandasaali, Karunkuruvai, Karuppu kavuni, Kattuyanam, Kothamalli samba, Kullankar, Kuruvai kalanjium, Kuzhiyadichan, Mapillai samba, Mottakur, Nootripathu, Paalthondi, Rangoon samba, Samba mosanam and Vellaigundu samba changed the KOH solution to red colour (Table 1). Contrarily, Anaikomban, Arasamba, Atturkichili samba, Kallukar, Katta samba, Korangu samba, Kudhiraivaali samba, Maranel, Milagu samba, Muthuvellai, Naatu basmati, Nalannamak, Palkachaka, Ponmani samba, Purple puttu, Seeraga samba, Sembuli samba, Sivan samba, Sornamasuri, Thanga samba, Thengaipoo samba, Thooyamalli, Thulasivasanai, Uppumolagi and Vellai kavuni changed the KOH solution to light yellow colour (Fig. 1). Similar kind of results were observed in rice

0. Control, 1. Sorna masuri, 2. Kattasamba, 3. Thulasi vaasanai, 4.Thanga samba, 5. Illupaipoo samba, 6. Salem sanna



0. Control, 1. Idly, 2. Vadakathi samba, 3. Mysore malli, 4. Sorna, 5. Samba mosanam, 6. Kattanur, 7. Vaasanaiseeraga samba



0. Control, 1. Poongar, 2. Mottakur, 3. Kothamalli samba, 4. Kullankar, 5. Nootripathu, 6. Kuruvaikalanjium, 7. Kallundrikar

Figure 1. Response of the rice varieties to NaOH and KOH tests

[9, 30-34], sorghum [24], wheat [35], Indian mustard [26] and chilli [4]. The colour formation process in KOH test is the same as that of NaOH test, the red pigment on the grains causes red colour change. This KOH test could be used for the identification of red and white kernelled varieties. Thus, the NaOH and KOH tests are mainly based on the colour reaction with pigment present on the grain. Therefore, if any white grain off-type seed admixted with coloured grain varieties, the chemical tests can be used to find out those admixture. Vice versa, any coloured grain rice present in white rice varieties, the NaOH and KOH tests can also be used.

In the present investigation, the NaOH and KOH test results showed that the red and white kernelled varieties can easily be differentiated. In addition to that, the absorbance of the coloured solutions was specific to the variety. Among the varieties, maximum absorbance was recorded in a red kernelled variety Kullankar (2.976 OD) and minimum in white kernelled variety Arasamba (0.502 OD) in NaOH test. Likewise, Norungan (2.947 OD) and Korangu samba (0.135 OD) recorded the highest and lowest absorbance's, respectively due to KOH reaction. This indicates that the red kernel nature has given higher absorbance and white kernel variety with lower absorbance. Thus, the varietal identification can also be done by measuring the absorbance of the coloured solutions from NaOH and KOH tests since every variety has a specific absorbance value.

In addition, the colour intensity of the solutions from NaOH and KOH tests were measured and it is also specific to the varieties. In NaOH test, maximum colour intensity was observed in Paalthondi (99.7 ΔE) and minimum in Rajamannar (89.2 ΔE) and these varieties have red and white kernels. Likewise, the varieties Kandasaali (98.1 ΔE) and Sivan samba (88.2 ΔE) had the highest and lowest colour intensity in KOH test when measured in tintometer.

The cluster analysis performed using the OD values obtained from NaOH and KOH tests using eucledian distance and average linkage (between groups) formed five major clusters with 18, 28, 12, 2 and 5 varieties in cluster 1, 2, 3, 4 and 5 respectively (Fig. 2). Maximum varieties constitute cluster 2 followed by clusters 1, 3, 5 and 4. Cluster 4 constitutes varieties having OD value of 2.4 and 2.8 in NaOH and KOH tests. Cluster 1 contains varieties having OD values in the range of 1.5 to 2.3 in NaOH tests and cluster 2 is grouped by varieties having OD value in the range of 0.5-1.9 in NaOH test and cluster 3

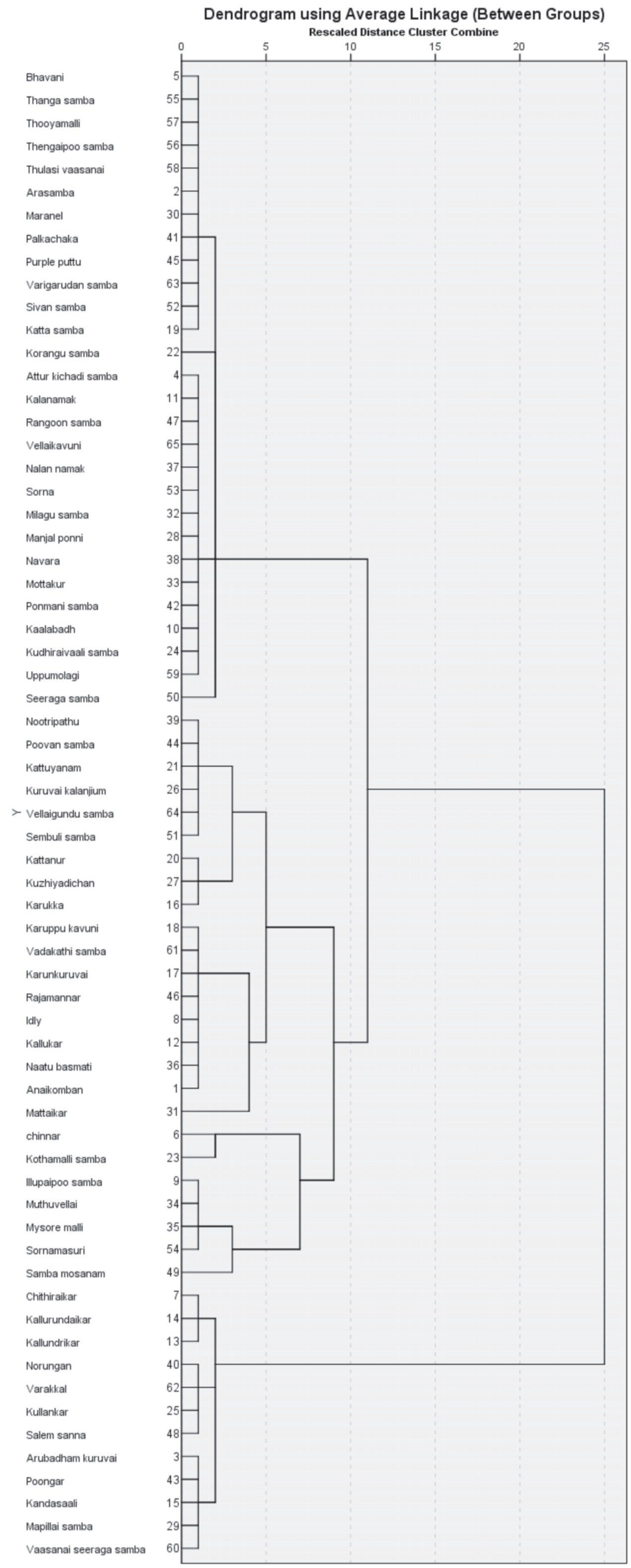


Figure 2. Dendrogram depicting cluster analysis of the OD value obtained using NaOH and KOH tests

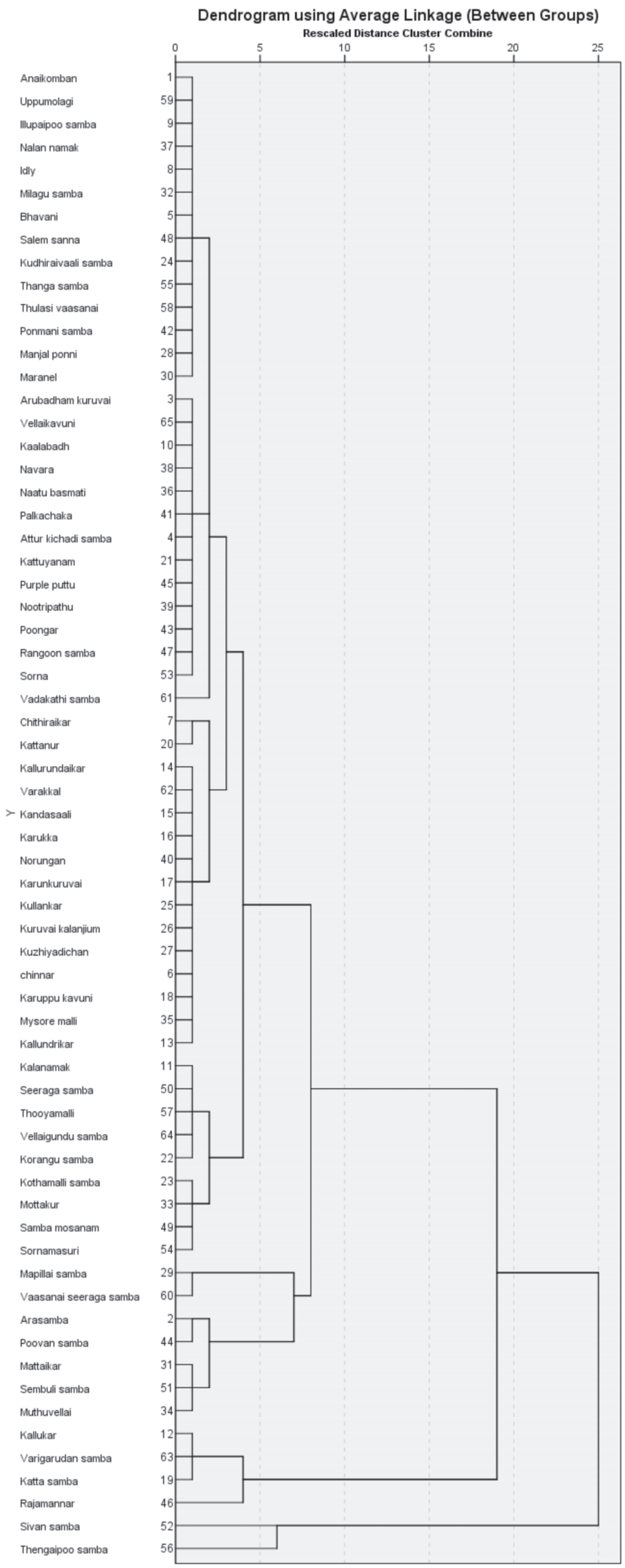


Figure 3. Dendrogram depicting cluster analysis of the “E value obtained using NaOH and KOH tests

constitutes varieties having OD values between 2.1-2.9. In addition, the inter cluster distance indicates that the varieties that are more similar have less distance and dissimilar varieties had wider distance.

The cluster analysis performed using the “E values showed five major clusters with 52, 5, 4, 2 and 2 varieties in cluster 1, 2, 3, 4 and 5 respectively (Fig. 3). Maximum varieties constitute cluster 1 followed by clusters 2, 3, 4 and 5 respectively. Cluster 4 is characterized by the varieties having ΔE values ranging from 98 to 99 from in NaOH test and 97 in KOH test. Cluster 5 contains varieties having ΔE values of 95 in NaOH and 88 to 91 in KOH tests. Cluster 3 contains varieties having ΔE values ranging from 89 to 91 in NaOH tests. The varieties in cluster 2 have ΔE values in the range of 97 to 99 in NaOH and 93 to 95 in KOH tests. The varieties having ΔE values in the range of 95 to 97 and 94 to 97 in NaOH and KOH tests are grouped under cluster 1. Clustering helps us in identifying genotypes that are similar in their characters and aids in the crossing program. The varieties belonging to clusters that are farther apart should be selected for crossing in order to prevent homogeneity.

CONCLUSION

The NaOH and KOH tests can be used to differentiate the varieties based on the colour of the kernel. Also, these tests can be applied wherein the other tests were not found useful for differentiation or can be combined with other chemical tests for easy identification and characterization. Additionally, measurement of colour absorbance through UV-Visible Spectrophotometer and colour intensity by using Tintometer can be used as supplementary methods for obtaining the differences in the varieties. Further, the variations in the colour intensity based on the pigments present in the seed may be studied in future to make the groups.

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