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Identification of rice varieties through chemical tests

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ABSTRACT Variety identification has great significance from seed production, breeding as well as intellectual property rights point of view to ensure quality seed. Four varieties of rice were identified on the basis of seed colour (phenol, modified phenol and NaOH) and seedling response (GA_3 and 2, 4-D) to chemical tests. Though no individual chemical test was able to distinguish all the varieties, different chemical tests in conjunction were useful in identification of varieties. Karma Mahsuri exhibited negative response to phenol test and positive response to modified phenol test. However, three varieties namely, Danteshwari, Samleshwari and Mahamaya recorded positive response to both phenol and modified phenol test. Varieties Karma Mahsuri, Samleshwari and Mahamaya showed yellow colour reaction with NaOH test while, Danteshwari showed yellowish brown reaction. The effect of GA_3 and 2, 4-D on coleoptile growth of seedling was found to be variable among varieties studied.

Key Words: Standard phenol, modified phenol, NaOH, GA_3, 2, 4-D, varietal identification

Characterization of cultivars, establishment of varietal identity and genetic purity of the seed lots are crucial for varietal improvement, varietal protection and seed production. A rapid and reliable technique to verify the identity and to assess the purity of seed lots is important in seed quality assurance programme to meet out the minimum seed certification standards of seed quality prescribed for the certified class. Hence, in order to establish and maintain a high market reputation, all major seed companies, both in private and public sectors, take necessary care to ensure highest quality standards. Verification of the seed purity is essential before marketing of commercial seed. To ensure the standards of genetic purity, grow-out test is being conducted (GOT), where purity level is assessed through morphological characteristics at various stages of plant growth. However, GOT is based on the set of characters that are influenced by environment to some extent and requires more time. This drawback and recent innovations in the molecular markers had highlighted the need to investigate other non-conventional methods for testing of purity. The seed industry is under pressure to use rapid techniques for testing genetic purity. In the present study four varieties released from India Gandhi Agricultural University, Raipur was involved to characterize based on morphological, chemical and molecular markers.

Together with the quick and simple method for DNA extraction, SSR and ISSR analyses were proved to be a promising approach in variety authentication and purity monitoring. This marker technology will provide corporate managers and breeders with legal evidence of commercial varietal seeds in the seed market and will help to protect plant proprietary rights of the rice varieties. The only legally recognized traditional method in our country for cultivar identification and genetic purity assessment continues to be seed certification based on grow-out tests, which include only morphological characteristics of a variety. Such methods have been highly successful and efficient, but concerted efforts of plant breeding programme tend to produce new varieties, which are less phenotypically distinct due to closely related genetic population used in variety development programmes.

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The morphological traits may not be sufficient for discrimination and identification of all the extant and new varieties, warranting more precise technique. As a result, there is a greater need to explore alternative methods to characterize crop cultivars. Developments of various techniques in order to identify the varieties in the laboratory are beset with many difficulties and a single technique may not be adequate correctly to identify a variety. 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 of various techniques has been employed for identification of varieties.

MATERIALS AND METHODS

Four rice varieties released from IGKV, Raipur, namely, Karma Mahsuri, Danteshwari, Samleshwari and Mahamaya were considered for the present study. The nucleus seed of these four varieties were taken.

Identification and grouping of rice genotypes through chemical tests

The study included the following chemical test:

Phenol test

The phenol test for varietal purity testing as suggested by Walls [1] was followed. Four replications of 100 seeds were soaked in distilled water for 24 hours. The seeds were then placed in petri dishes containing filter paper moistened with 5 ml of 1% phenol solution and kept at room temperature (28°C) for 24 hours. After that, the seeds were examined and grouped into different colour classes as no colour change, light brown, brown and dark brown.

Modified phenol test with copper sulphate or ferrous sulphate

As described by Banerjee and Chandra [2], the procedure was similar to the standard phenol test except that the seeds were soaked in a solution of 0.5% $CuSO_4$ or $FeSO_4$ instead of soaking seeds in distilled water. The seeds were examined and grouped into five distinct groups namely, no colour change, light brown, brown, dark brown and black.

NaOH test

Four replications of fifty seeds each were soaked in 3% NaOH solution for 3 hours and thereafter, the change in colour of the solution was observed. Based on intensity of colour reaction, the genotypes were classified into three groups *viz.*, no colour change, light yellow and wine red.

Seedling growth response to GA3

The seeds surface of rice genotypes were sterilized by washing in distilled water. Fifty seeds each in three replications were placed on two layers of blotter paper moistened with 25 ppm GA_3 solution and incubated at 25 ± 10 °C as per ISTA procedure. The water soaked blotter papers were used as the control. On seventh day, the coleoptiles length of twenty five randomly selected seedlings was measured and the growth response was recorded as percent increase in coleoptilelength over control. The percent increase in coleoptile length over control was calculated using the following formula....

Percentage increase over control= Difference / Control (G%) X 100

The genotypes were grouped based on percent increase of coleoptiles length over control as follows:

a) Very low response : < 10 % increase

b) Low response : 10-30 % increase

c) Moderate response: > 30 % increase

Seedling growth response to 2, 4-D

The seeds surface of rice genotypes were sterilized by washing in distilled water. Fifty seeds each in three replications were placed on two layers of blotter paper moistened with 5 ppm of 2, 4-D solution and then incubated

at 25±10 °C as per ISTA procedure [3]. The water soaked blotter papers were used as control. On seventh day, coleoptile length of twenty five randomly selected seedlings was measured and the sensitivity response of genotypes was recorded as percent decrease in coleoptile length over control. The decrease in coleoptile length over control was calculated using the formula such as:

$$\text{Percent decrease over control} = \frac{\text{Increase over coleoptile in 2, 4D}}{\text{Coleoptile length in control}} \times 100$$

The genotypes were grouped based on percent decrease of coleoptile length over control as follows:

a.) Susceptible : < 85 %

b.) Highly susceptible : > 85 %

RESULTS AND DISCUSSION

Developments of various techniques in order to identify the varieties in the laboratory are beset with many difficulties and a single technique may not be adequate correctly to identify a variety. 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 of various techniques has been employed for identification of varieties.

Phenol test

The phenol colour reaction revealed that the rice genotypes could be grouped into two colour groups. Out of four rice varieties, Karma Mahsuri exhibited no colour change (-) and rest of the three genotypes Danteshwari, Samleshwari and Mahamaya showed light brown colour (++) (Table 1, Fig. 1). Phenol colour reaction appeared to be an important diagnostic character, due to that fact that it is based upon the activity of tyrosinase, whose presence is under genetic control. Moreover, the differences in colour reaction are attributed to the level of polyphenol oxidase on seed surface.

Modified phenol colour test

The modified phenol test using $FeSo_4$ solution helped in further sub-division of standard phenol groups [4]. Varieties Danteshwari, Samleshwari and Mahamaya showing brown colour in phenol test had dark brown colour in modified phenol colour test. While, Karma Mahsuri showed no colour change in phenol test had brown colour in modified phenol colour test (Table1, Fig.2).

Sodium hydroxide (NaOH) test

Varied response of rice varieties to sodium hydroxide test was observed (Table 1, Fig. 3). Based on the colour development of the decanted solution, the varieties were grouped into two groups' *viz.*, yellowish brown and yellow. Among four varieties the response of three varieties namely, Karma Mahsuri, Samleshwari and Mahamaya was yellow whereas, it was yellowish brown in Danteshwari.

Coleoptile growth response to gibberellic acid (GA)

The coleoptile length of rice genotypes showed varied response to GA_3 (Table 2, Fig. 4). The highest increase in coleoptile length was observed in Karma Mahsuri (79.74 percent) and the lowest was in Samleshwari (46.47 percent). Based on the percent increase in coleoptile length over control, all the varieties exhibited moderate response to gibberellic acid. Danteshwari and Mahamaya had 46.57 and 79.46 percent increased coleoptile length over control, respectively.

Coleoptile growth response to 2, 4-D

The coleoptile of rice genotypes showed varied response to 2, 4-D (Table 3, Fig. 4). The mean length of coleoptile under control and at 2, 4-D was 4.91cm and 3.32 cm, respectively. The highly affected genotype was Danteshwari (41.09 %) and the least affected was Karma Mahsuri (17.32 %). Based on the percent decrease in coleoptiles length over control, all the four varieties were grouped into susceptible (< 85%) category.

Table 1. Identification of varieties based on Chemical test

S.N.	Chemical Tests	Varieties			
		Karma Mahsuri	Danteshwari	Samleshwari	Mahamaya
1.	Phenol test	No colour change	Brown	Brown	Brown
2.	Modified Phenol test	Brown	Dark Brown	Dark Brown	Dark Brown
3.	NaOH test	Yellow	Yellowish brown	Yellow	Yellow

Table 2. Identification and grouping of Rice varieties based on coleoptiles growth response to GA3

Varieties	Coleoptiles growth (cm)		Percent increased over control	Groups
	Control	GA_3		
Karma Mahsuri	3.68	6.62	79.74	Moderate
Danteshwari	5.61	8.23	46.57	Moderate
Samleshwari	5.23	7.66	46.47	Moderate
Mahamaya	5.13	9.20	79.46	Moderate
Mean	4.91	7.93	63.06	Moderate

Very low response : < 10 %
Low response : 10-30 %
Moderate response : > 30 %

Table 3. Identification and grouping of Rice varieties based on coleoptiles growth response to 2, 4-D

Varieties	Coleoptiles growth (cm)		Percent decreased over control	Groups
	Control	2,4-D		
Karma Mahsuri	3.68	3.04	17.32	Susceptible
Danteshwari	5.61	3.30	41.09	Susceptible
Samleshwari	5.23	3.20	38.67	Susceptible
Mahamaya	5.13	3.74	26.98	Susceptible
Mean	4.91	3.32	31.02	Susceptible

a.) Susceptible : < 85 percent

b.) Highly susceptible: > 85 percent

The most practiced approach to varietal identification involves the study of morphological characteristics through grow out test (GOT). For rice (*Oryza sativa* L.), 62 morpho-physiological characteristics have been described under DUS guidelines in India [5]. Though, morphological traits are simple and irreplaceable, these descriptors suffer from many drawbacks, such as influence of environment on trait expression, epistatic interaction and pleiotropic effects [6]. Furthermore, paucity of sufficient number of stable morphological markers for unequivocal identification of increasing number of reference collection of varieties enforces to look for alternatives.

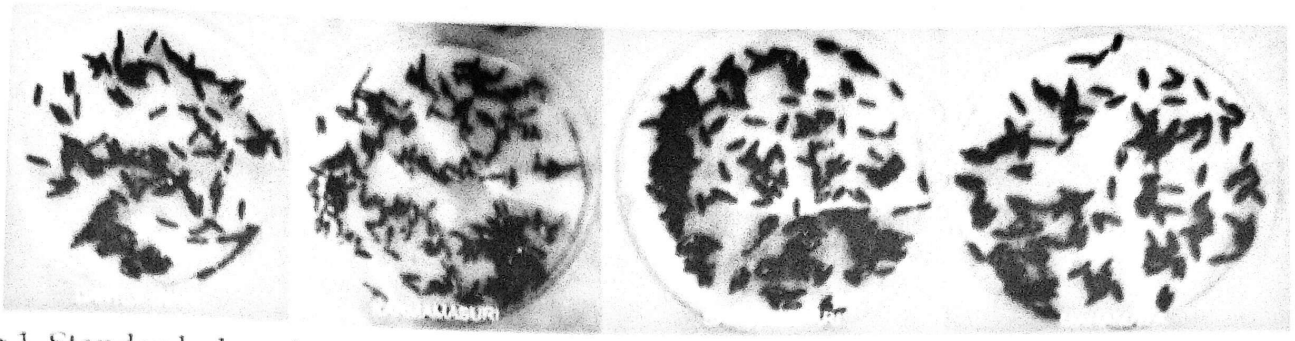


Fig.1. Standard phenol colour test

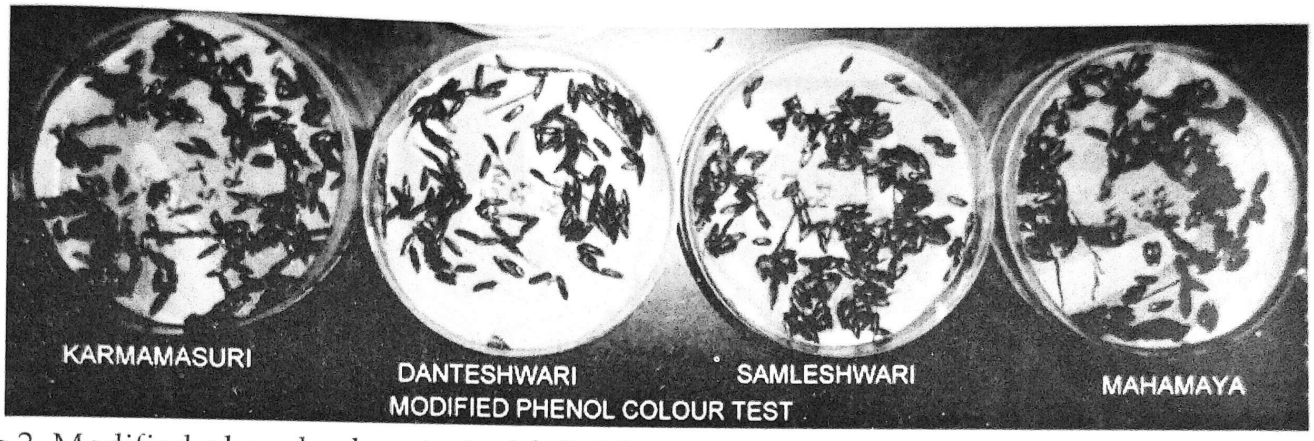


Fig.2. Modified phenol colour test with $FeSO_4$

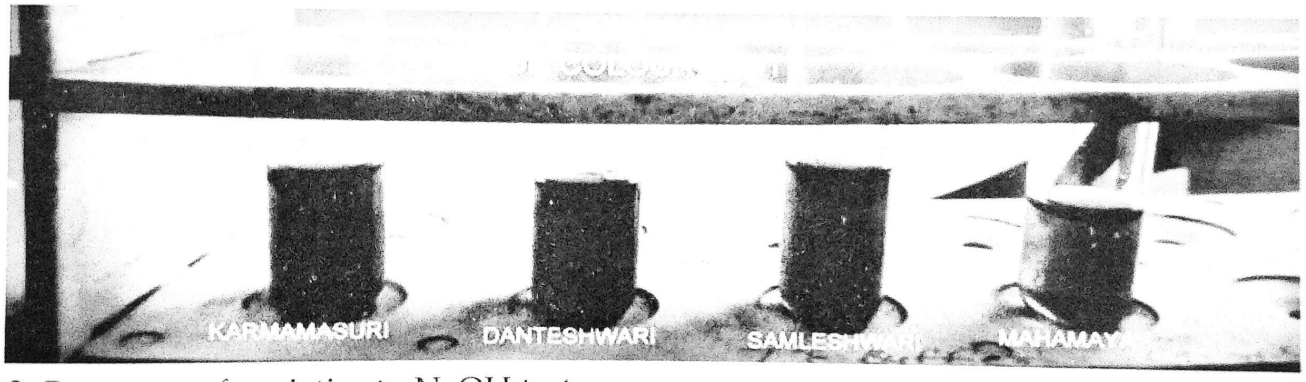
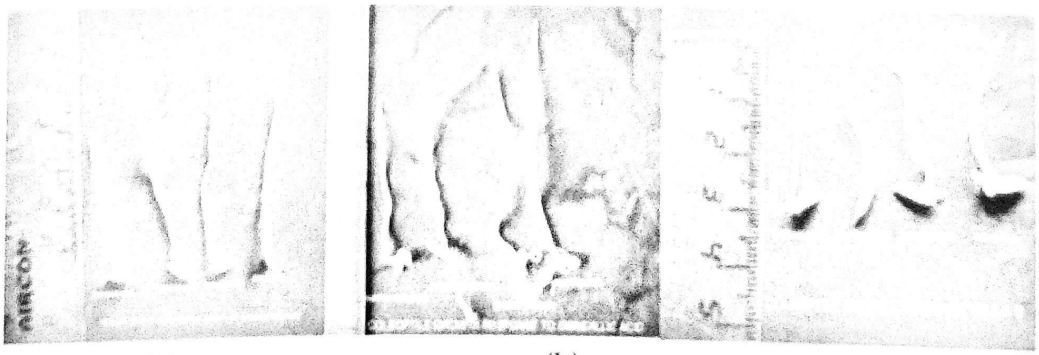


Fig.3. Response of varieties to NaOH test



(a) (b) (c)

a). Seedling response in control b.) Seedling response to GA_3 c.) Seedling response to 2, 4-D

Fig.4. Response of varieties to GA_3 and 2, 4-D tests

Chemical tests are quick, easy and reproducible and adopted in several crops [7]. A number of chemical tests have been developed for varietal identification such as phenol test [8-9], modified phenol test [9], sodium hydroxide test, gibberellic acid response test and 2, 4-D soak test [7]. Very often these tests provide supportive evidence for morphological evaluation of seeds [10].

Out of four varieties subjected to phenol test, one variety showed no colour change and three showed brown colour. Under modified phenol test, one variety Karma Mahsuri showed brown reaction while, three showed dark brown reaction. The results obtained are in agreement with findings of [4, 11].

Sodium Hydroxide test did not show any differences in colour of solutions excluding Danteshwari which showed yellowish brown colour reaction. Similar results were also reported by Rao *et al.* [12].

The seedling response to GA_3 and 2, 4-D gives useful information in identifying the genotypes in many crops [7, 13-14]. The coleoptile growth response of rice genotypes to gibberellic acid and 2, 4-D were studied. The percent increase in coleoptile length over control was to the tune of 79.74 percent (Karma Mahsuri), 79.46 percent (Mahamaya), 46.57 percent (Danteshwari) and 46.47 percent (Samleshwari). Similarly the percent decrease in coleoptile length was to the tune of 41.09 percent (Danteshwari), 38.67 percent (Samleshwari), 26.98 percent (Mahamaya) and 17.23 percent (Karma Mahsuri).

Seed based techniques for varieties are warranted for rapid identification of seed lots. Therefore, in the present study, a strategy of using seed based chemicals tests in development of seed keys for rice varieties was demonstrated in four varieties developed by IGKV, Raipur.

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