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RESEARCH ARTICLE :

Characterization of parents and F_1 seeds of rice hybrid KRH-4 using different chemical tests and seed image analyzer

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SUMMARY : Investigations were carried out to study the varietal characterization of paddy hybrid and its parents through chemical tests and using iamge analyzer. Among the different chemical tests, phenol and modified phenol test showed differences in which male seeds had no change in colour development whereas, seeds of female and hybrid showed brown colouration. In case of $FeSO_4$, KOH and NaOH tests both parents and hybrid showed similar colour reaction. The results obtained through image analysis with regard to seed length and seed width could be useful for varietal characterization of paddy genotypes. Based on seed length both parents and hybrid were grouped as medium length, based on seed width, parents and hybrid were grouped as broad seeds. But based on Seed length/ width ratio of seeds, female and hybrid seeds were grouped as elongated seeds whereas, male as semi elongated.

KEY WORDS :
Hybrid, Paddy, Colour

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BACKGROUND AND OBJECTIVES

Rice has a renowned relationship with the humans since ages. Presently, more than half of the world's population depends on rice as a staple food. Asia can be considered as 'Rice Basket' of the world, as more than 90 per cent of the rice is produced and consumed in Asia, a region with high population density. Genetically divergent rice genotypes are available due to diverse conditions of their cultivation and hence it is very difficult to visually identify cultivars on the basis of phenotypic characters. At present, large number of varieties and hybrids with special regard to yield, seed quality, resistant to biotic and abiotic stresses are under cultivation throughout the country. In recent years public institutions and private companies introduces one after the other hybrids/varieties for commercial cultivation. At present, large number of varieties and hybrids with special regard to yield, fruit quality, resistant to biotic and abiotic stresses are under cultivation throughout the country. The present trend of

continuous release of rice varieties from Central and State Varietal Release Committee has warranted to develop suitable techniques for varietal identification at the laboratory level particularly when the seeds have been submitted for seed purity analysis. Maintenance of genetic purity of varieties is of primary importance for preventing varietal deterioration during successive regeneration cycles and for ensuring varietal performance at an expected level. The chemical tests reveal differences among the seeds and seedlings of different varieties. These tests require virtually no technical expertise or training and can be completed in a relatively short time. The results of these tests are usually distinct, easily interpreted and help in grouping of the genotypes. Characterization of genotypes assumes importance with the implementation of Protection of Plant Varieties and Farmer Rights Act (PPV and FRA). Seed shape and size are among the most important agronomic traits because they affect yield, eating quality and market price. In general, seed shape can be scored in two ways. The simple way is to measure seed length (L) and width (W) with calipers. However, manual methods have limits to the number of data, the quality of measurements, and the variety of shape data that can be gleaned. By contrast, computational methods using digital imaging technology could enable us to automatically measure a variety of shape parameters at very small sizes in high-resolution images. Recently, image analysis application (IAA) has been widely used in studies of size, shape and color measurements of rice. Therefore, an investigation was carried out to study the response of rice genotypes to various chemical tests to explore the possibility of using these tests for determination of cultivar purity in rice.

RESOURCES AND METHODS

Chemical tests :

Phenol test:

Four replications of 25 seeds were presoaked in distilled water for 24 h at 25° C. Then they were transferred on to two layers of Whatman No.1 filter paper saturated with one per cent phenol solution. The petri dishes were covered and incubated at 25° C and the colour reactions were noted after 24 h. Based on the development of seed coat colour, the selected cultivars were classified into different categories as No change in colour, Light brown, Brown, Dark brown, Light grey, Grey and Dark grey.

Modified phenol test:

Modified phenol test was conducted similar to standard phenol test exceptthat four replications of 25 seeds seeds were soaked either in 0.5 per cent $CuSO_4$ or one per cent $FeSO_4$ solution for 24 h instead of distilled water. Colour reaction was noted after 48 h of incubation and the cultivars were classified based on colouration of seed coat into different categories as no colour change, light brown, brown, dark brown, light grey, grey and dark grey.

NaOH test:

Four replications of 25 seeds were soaked in two per cent NaOH solution for one hour and thereafter change in colour of the solution was observed. Based on the intensity of the colour reaction, the genotypes were classified into two group's *viz.,* no change in colour, yellow and light yellow.

KOH test:

Four replications of 25 seeds were soaked in four per cent KOH solution for three hours and thereafter change in colour of the solution was observed. Based on the intensity of the colour reaction, the genotypes were classified into two group's *viz.,* no change in colour and reddish brown.

Ferrous sulphate test :

Four replications of 25 seeds ofeach genotype were soaked in 50 ml of 0.5% $FeSO_4$ solution for 4 h at 25°C. Based on the seed color, development varieties were grouped as grey, light grey, dark grey and black.

Peroxidase test :

It was studied as per the procedure given by Buttery and Buzzell (1968). Ten seed coats were removed and placed separately in the test tube, with three replications for parents and hybrid and added 10 drops of 0.5 per cent Guaiacol solution into test tube, after ten minutes one drop of 0.1 per cent solution of hydrogen peroxide (H_2O_2) was added and the reactions were noted exactly after sixty seconds. The colouration due to peroxidase activity was observed to group the varieties as,

- Present: brown colour solution
- Absent: colourless solution

Seed morphological characterization using seed

image analyzer :

Biovis is a seed image analyzer of new generation, based on digital image processing technology, easy to handle and to operate. After irregularly distributing a seed sample on special measuring plane (image scanner), it takes a picture of the objects to be explored under optimal lighting conditions. This image is evaluated in the PC by special software on the basis of the digital image processing. Seeds of different shape and size will be counted fast and reliably, the measurements of their lengths and widths will be determined as well as their size infractions. The features of varieties may be stored in a pre-configured, able to learn data base for recognizing and identifying main seeds, foreign seeds and non-seed particles. The analysis data are entered and reported together with user-selectable statistical information in a measuring protocol. Furthermore it is possible to transfer specific measuring data to a user-specific database. The Biovis is a device comprises a scaner, which scan the images and capture the same with their length, width, area, perimeter and roundness with coloured images. Thus it is suitable for the analysis of the seeds of many crops. The seed image analyzer Biovis provides clearly more than a traditional counting device. It works fast, noiseless and easily operated. The special Biovis image anlysis software offers a lot of possibilities, to adjust the recording of the analysis results and statistical interpretation according to the requirements and demands of the user.

Test weight :

Four replications of 1000 seeds of each genotype were counted randomly and weighed upto two decimal places. The mean of 1000 seed weight was expressed in grams and genotypes were classified into three groups which are as follows:

- Very low (<15 g)
- Low (15-20 g)
- Medium (21-25 g)
- High (26-30)
- Very high (>30 g)

Seed width (mm) :

100 seeds of each parent and hybrid were measured using Biovis image analyzer and grouped as below,

- Very narrow (<2.0 mm)
- Narrow (2.1-2.5 mm)
- Medium (2.6-3.0 mm)
- Broad (3.1-3.5 mm)
- Very broad (>3.5 mm)

Seed length :

100 seeds of each parents and hybrid was measured using biovis image analyzer and were grouped as,

- Very short (<6.0 mm)
- Short (6.1-8.5 mm)
- Medium (8.6-10.5 mm)
- Long (10.6-12.5 mm)
- Very long (>12.5 mm)

Seed shape :

100 seeds of each parents and hybrid was measured using biovis image analyzer and were grouped as,

- Short slender
- Short bold
- Medium slender
- Long bold
- Long slender
- Extra long slender

Seed colour :

The seed colour was observed under natural day light conditions and classified in to groups,

- Brown
- Yellow
- Golden
- Straw

OBSERVATIONS AND ANALYSIS

The results obtained from the present study as well as discussions have been summarized under following heads:

Chemical tests :

Varietal identification by morphological characters is laborious, time consuming, tedious, cumbersome and costly affairs. A number of chemical tests have been developed for varietal identification such as phenol test, ferrous sulphate test, potassium hydroxide test, sodium hydroxideand peroxidase test. These chemical tests are very quick, easy and reproducible (Ashwani Kumar *et al.*, 1995). Very often these tests provide supportive evidence for morphological evaluation of seeds (Vanderburg and Vanzwol, 1991) and aid in preparation

of varietal identification keys.

On the basis of seed colouration with phenol test, paddy hybrid KRH-4 parents were grouped into three categories (light brown, brown and dark brown), female seeds showed brown colour, male seeds not shown any colour change and hybrid showed dark brown colour.

Seed colouration with phenol is one of the important qualitative characteristic which is not affected by environmental condition. The result of phenol test (Fig. 1) is usually distinct and easily interpreted. Walls (1965) reported that the phenol colour reaction depends on the quality and quantity of oxidase enzymes present in seeds. Whereas, Takahashi and Hamza (1983) reported monophenol oxidase was extremely localized in grains even though it is present in all other plant parts. Phenol colour reaction, which is an index of polyphenol oxidase activity, has been utilized to distinguish the crop varieties by earlier workers (Joshi and Banerjee, 1970) in wheat and Sparks *et al*. (1985) in rice.

The potassium hydroxide test is useful in determining the varietal difference based on the chemical reaction. On the basis of colour reaction with KOH solution both parents and hybrid shown yellow coloured reaction with KOH test. Similar groupings were reported by Sambasiva Rao *et al*. (2002) in groundnut and Biradar Patil *et al*. (2006) in safflower genotypes. Varied colour reaction may be due to the chemical composition of seed or selective action of enzymes present which may be

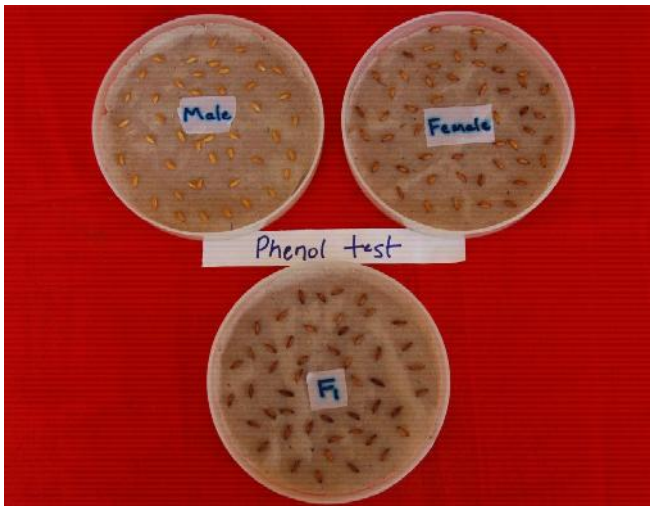

Fig. 1 : Phenol test for identification of parents and hybrid

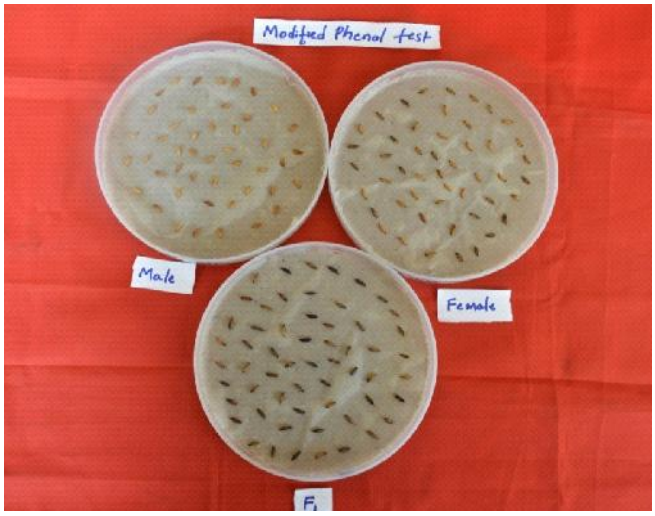

Fig. 2 : Modified phenol test for identification of parents and hybrid

Table 1 : Response of parents and hybrid to different chemical tests in rice hybrid KRH-4

Chemical test	Female	Male	Hybrid
Phenol test	Brown colour	No colour change	Dark brown colour
$FeSO_4$ test	Brown streaks	Brown streaks	Brown streaks
KOH test	Yellow	Yellow	Yellow
NaOH test	Light brown	Light brown	Light brown
Modified Phenol test	Brown colour	No colour change	Dark brown colour
Peroxidase test	No colour change	No colour change	No colour change

Table 2 : Chacterization of parents and hybrid using seed image analyzer

Parameters	Female	Male	Hybrid
Test weight (g)	16.29	17.86	16.56
Seed width (mm)	3.23	3.30	3.19
Seed length (mm)	10.38	9.63	10.45
Seed length/ width ratio	3.21	2.91	3.27
Seed shape (mm)	Short slender	Short bold	Short slender
Seed colour	Yellow	Golden yellow	Straw

governed genetically.

Both parents and hybrid shown light brown colour reaction with sodium hydroxide solution. Similar classification by NaOH test was reported earlier by Sambasivarao *et al*. (2002) in rice, Ponnuswamy *et al*. (2003) in cotton and Biradar Patil *et al*. (2006) in safflower genotypes. The colour reaction to sodium hydroxide solution was obtained due to reaction of seeds to secondary metabolites (Vanderburg and Vanzwol, 1991).

The parents and hybrids showed negative reaction to peroxidase activity. The results of peroxidase test are not in conformity with previous work reported by Ashwani kumar *et al.* (1995) (Table 1).

In ferrous sulphate test, parents and hybrids showed brown streaks; similar results were also reported by Saharan (1991) in paddy and Ponnusamsy *et al*. (2003) in cotton.

Seed morphological characteristics using seed image analyser (Biovis) :

On the basis of variation in seed morphometric characteristics *i.e.*, seed length and width assessed through image analysis were grouped and varietal identification keys were prepared. By image analysis, based on seed length parents and hybrids classified as medium length, both parents and hybrids have broad seed width based on seed length/ width ratio, female and hybrid classified elongated where male seeds classified as semi elongated.

Based on seed shape, female and hybrid classified as short slender and male as short bold. There was no difference in case of test weight. Both parents and hybrid grouped as light weight seeds. Based on colour, male seed possess golden colour, female as yellow colour and hybrid as straw coloured seeds (Table 2).

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