Comparative Evaluation of Different Sorghum (*Sorghum bicolor* L. Moench) Cultivars on the Basis of Chemical Tests

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Twelve varieties of sorghum (*Sorghum bicolor*) were studied by chemical tests *viz.* phenol and modified phenol test, $FeSO_4$ test, KOH-bleach test, and response of GA_3 and 2, 4-D. The varieties included were HC-136, HC-171, HC-260, HC-308, PC-1, PC-6, PC-9, PC-23, PC-121, SSG59-3, CSV-15 and MP-Chari. Based on phenol test sorghum varieties can be grouped into four categories *viz,* light brown (PC-6, PC-9), brown and black (SSG 59-8). In the case of modified phenol test, two groups were identified *i.e.* group A and group B, group A is characterized into dull grey, light grey, dark grey and brown colour and group B included light buff colour, yellow colour light brown, brown and brownish red. Seven varieties showed light grey colours, one variety (PC-6) showed yellow colour, two grey and two black colour in response to ferrous sulphate test. KOH-bleach test broadly divided these varieties into two groups, off white colour (seven varieties) and red colour (five varieties). Response to GA_3 and 2,4-D in comparison to control helped to differentiate the varieties from each other on the basis of effect on germination (plumule and radicle length).

Key Words: Ferrous Sulphate, Forage Sorghum, Phenol, Potassium Hydroxide

The importance of cultivar identification was recognized in the late 19th century and early 20th century when new field crop varieties often lost their identity due to their admixture with other varieties or general types. Hence, there is a need to develop rapid and accurate laboratory techniques which can supplement the plant morphology. The various laboratory techniques which are employed range from simple method of examining seed and seedling morphology to the more advanced procedure of analyzing DNA polymorphism (Smith and Smith, 1992; Cooke, 1995). Seed morphology studied by visual examination and under magnification (by scanning electron microscopy SEM) can provide useful information for differentiating genotypes (Bahadur *et al.,* 1994). Examination of seedling morphology under controlled conditions of growth can be useful and cost-effective for describing and distinguishing cultivars (ISTA, 1992). Practically, a variety should be distinct, uniform and stable (DUS) with respect to the characteristics for use in varietal identification. Therefore, it is of paramount importance for those engaged in quality seed production and certification that they should be well acquainted with the diagnostic characteristics of different varieties.

An attempt has been made in this paper to identify the important sorghum (*Sorghum bicolor*) cultivars by subjecting them to different chemical tests and preparing schematic flow diagrams.

Materials and Methods

Phenol Test

Seeds were soaked in water for 16-24 h and 50 seeds of each genotype were placed in 15 cm Petri dish on two layers of filter paper soaked in 1% phenol solution. The Petri dishes were immediately covered. Seed colour was observed after 8 and 24 h. The procedure for modified phenol test is similar as above except the seeds were pre-soaked in 0.4% solution of $CuSO_4$ and 0.6% solution of Na_2CO_3 for addition of Na^+ ions instead of water.

Modified phenol test A = Pre-soaking in 0.4% $CuSO_4$.

Modified phenol test B = Pre-soaking in 0.6% Na_2CO_3.

Ferrous-sulphate ($FeSO_4$) Test

Fifty seeds were soaked in 1% $FeSO_4$ solution and kept in an incubator for four hours at 25°C. Varieties were categorized on the basis of change in the colour of the solution, namely, yellow, light grey, grey and black.

Potassium-hydroxide (KOH) Bleach Test

The presence or absence of a darkly pigmented testa or undercoat layer can be used to help in differentiating sorghum cultivars. The dark pigment in the testa has been identified as tannic acid. The test was conducted by preparing (1:5 w/v) solution of potassium hydroxide and fresh house hold bleach (5.25% NaOCl). Seeds were put into a glass container and were completely covered with KOH-bleach solution and kept at room temperature for the test. The seeds with brown seed coat soaked for 10 min and the seeds with the white seed coat for 5 min. in this solution and the mixture gently swirled periodically. Then the seeds were transferred to a sieve and rinsed with tap water. The seeds were placed on a paper towel and allowed to

air dry. The dry seeds were not patted to avoid removal of the pigment. After the seeds had dried results were recorded (number of dark seeds and number of light seeds).

Gibberelic Acid (GA_3) Test

Twenty five seeds were sown in Petri dishes lined by two layers of filter paper moistened with GA_3 (100 ppm). The Petri dishes were kept in a germinator at 25°C. The data was recorded at regular interval of 2 days up to 10 days for both plumule and radicle length.

2,4 Dichlorophenoxy Acid (2,4-D) Test

Solution of 2,4-D (1 ppm) was used in this method. The method was similar to that used for GA_3 test. The effect of 2,4-D on different cultivars was seen as the response of plumule and radicle emergence.

Results and Discussion

Advantage of phenol test is that it serves as an easy, quick and reliable test for the identification of different genotypes. The study of phenol test revealed that sorghum varieties could be grouped into four types: very light brown (2 varieties), light brown (4 varieties), brown (5 varieties) and black (1 variety) and in case of modified phenol test-A, four distinct categories *viz,* dull grey (2 varieties), light grey (4 varieties), dark grey (3 varieties), brown (3 varieties) and in the case of modified phenol test-B also four categories *viz,* light buff colour (3 varieties), yellow colour (2 varieties), light brown (3 varieties), brown (3 varieties) and brownish red (1 variety) could be recognized, as shown in flow chart 1 and Table 1. Our results suggest that the modified phenol tests are better for the categorization of different varieties into different colour groups more critically. Banerjee (1974) studied 61 wheat varieties and grouped them into black, brown, light brown and negative on the basis of phenol reaction. Miyagawa and Yasue (1980) grouped rice into Japanese, Chinese and Indian on the basis of phenol colour reaction. Twenty-nine varieties of paddy were divided into different groups on the basis of phenol colour reaction (Gupta and Agrawal, 1988). The other eighty-five varieties of paddy were also studied and grouped into different colour categories (Vanagamudi *et al.,* 1988). Similar work has been done on wheat (Singhal and Prakash, 1988), rice (Chauhan and Nanda, 1984) and pearlmillet (Rao *et al.,* 1989).

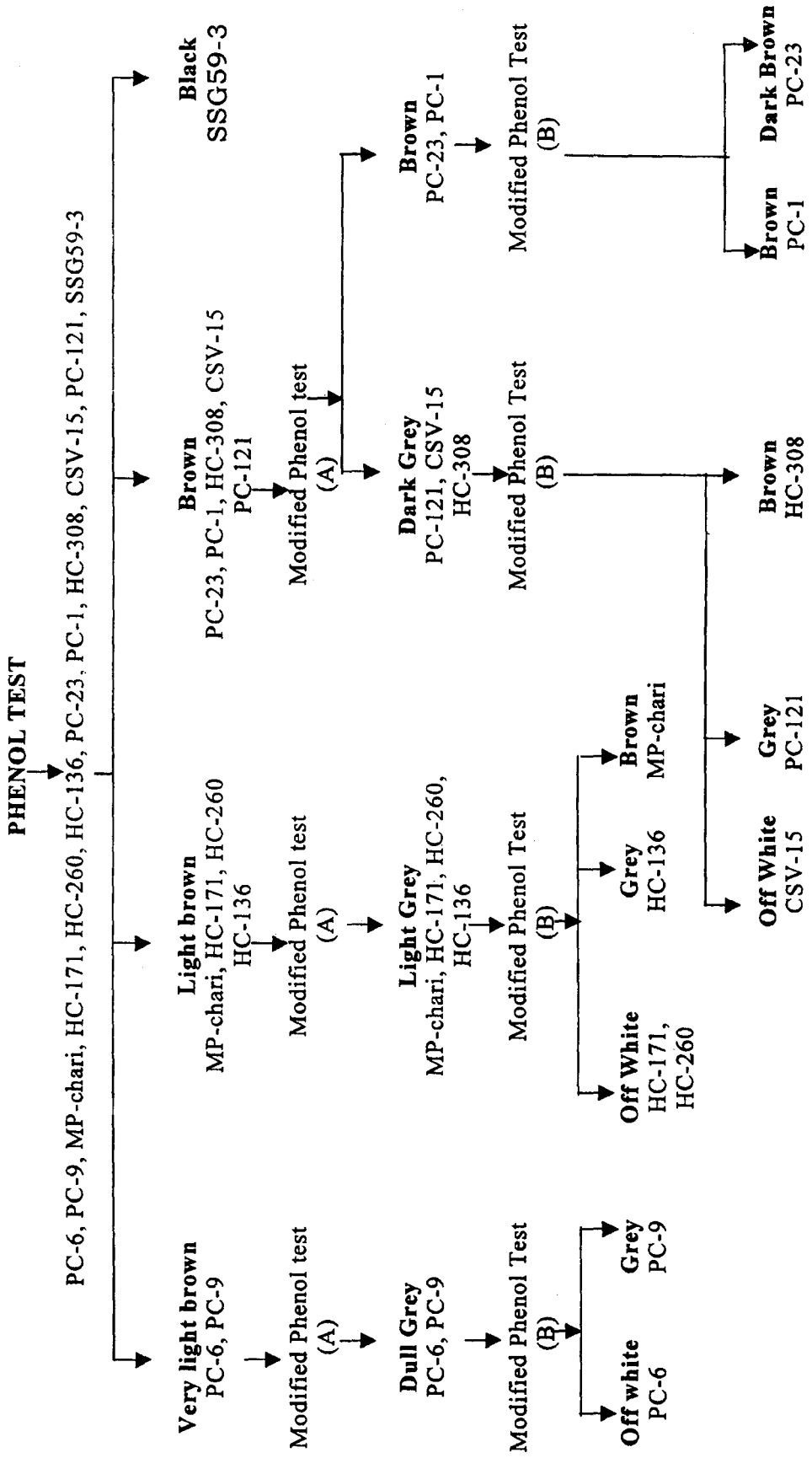
The use of ferrous sulphate colour reaction was also found to be a useful method in differentiating sorghum varieties (flow chart 2). Four different colour groups were made on the basis of $FeSO_4$ reaction. Seven varieties showed light grey colour, one variety (PC-6) showed yellow colour, two varieties showed grey and two varieties showed black colour (Table 1). Fe++ ions give different colour reactions with different genotypes in paddy husk. Saharan (1991) used the ferrous sulphate test in rice for varietal identification and reported 5 groups *viz.,* brown spots, brown streaks, grey, grey spot and grey streaks on kernels. Kumar (1992) applied $FeSO_4$ test in pearlmillet and grouped the hybrids into five colour groups.

On the basis of KOH test two major groups were identified. Seven varieties *i.e.,* HC-171, HC-171, HC-260, HC-308, PC-6, PC-9, CSV-15 and MP-Chari showed off white colour and five varieties *i.e.,* HC-136, PC-1, PC-23, PC-121 and SSG59-3 showed red colour reaction as shown in Table 1 and flow chart 1.

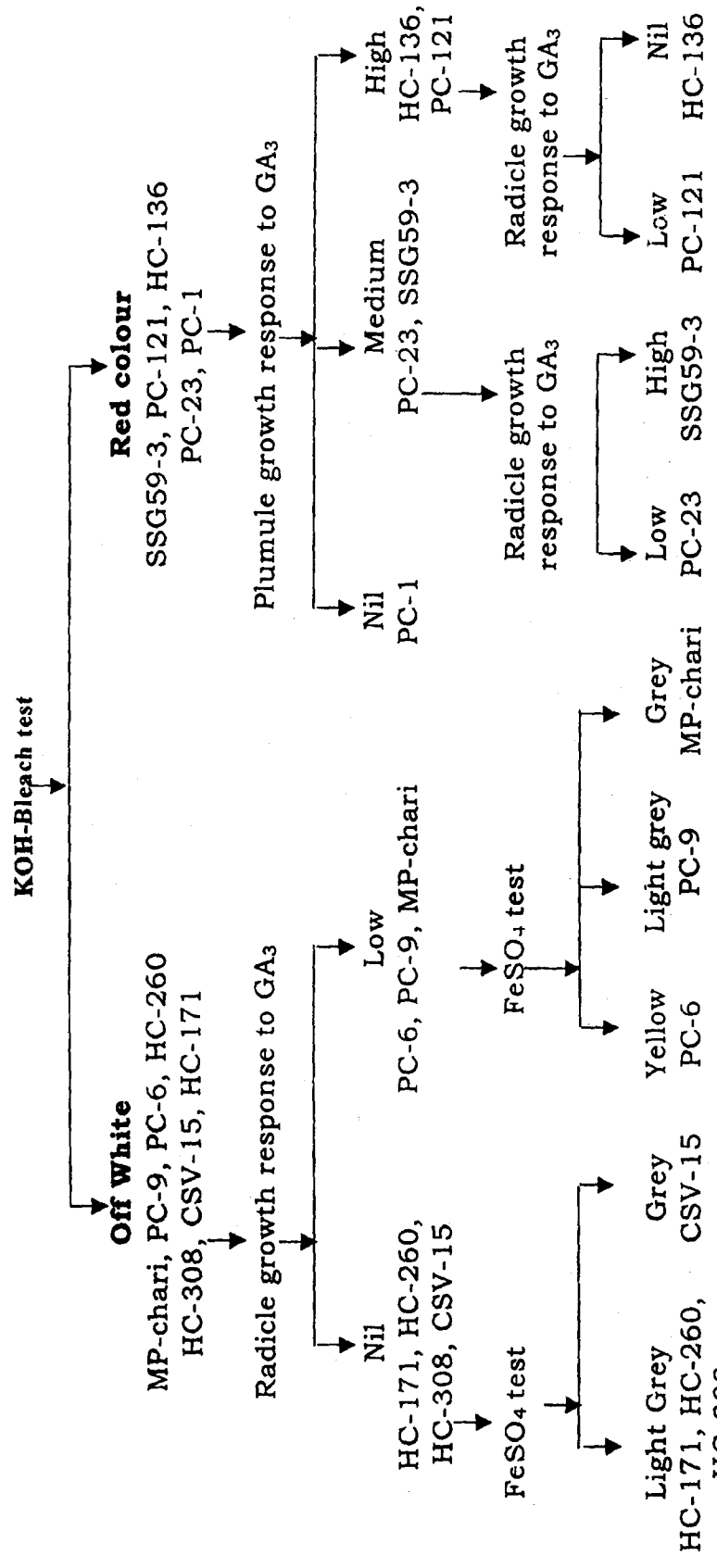
These varieties were classified into four groups on the basis of plumule and radicle growth response to GA_3 in comparison to control. The varieties were classified into four groups, as nil/no response (<10% increase over control), low (10-30% increase over control), medium (30-50% increase over control) and high (>50% increase over control) for both hypocotyl and radicle growth as

Table 1. Distinguishing sorghum varieties on the basis of chemical tests

Varieties	Phenol test	Modified Phenol test		Ferrous sulphate test	KOH-Bleach test
		(a)	(b)	$FeSO_4$	
HC-136	Light brown	Light grey	Brown	Light grey	Red
HC-171	Light brown	Light grey	Yellow	Light grey	Off white
HC-260	Light brown	Light grey	Light buff	Light grey	Off white
HC-308	Brown	Dark grey	Yellow	Light grey	Off white
PC-1	Brown	Brown	Brownish red	Light grey	Red
PC-6	V. Light brown	Dully grey	Light brown	Yellow	Off white
PC-9	V. Light brown	Dull grey	Light brown	Light grey	Off white
PC-23	Brown	Brown	Brown	Black	Red
PC-121	Brown	Dark grey	Brown	Light grey	Red
CSV-15	Brown	Dark grey	Light buff	Grey	Off white
SSG 59-0	Black	Brown	Light brown	Black	Red
MP-Chari	Light brown	Light grey	Light buff	Grey	Off white



Flow Chart 1. Varietal identification of sorghum on the basis of phenol and modified phenol test



Flow Chart 2. Varietal identification of sorghum on the basis of KOH-test $FeSO_4$-test, plumule and radicle growth response to GA_3

shown in Table 2 and flow chart 2. GA_3 is a growth regulator and stimulates the coleoptile growth whereas 2,4-D inhibits the root length. In case of 2,4-D test, all the varieties responded in the same manner *i.e.*, plumule were insensitive and root growth inhibited. Even in the presence of 2,4-D solution plumule were found to be least affected in comparison to radicle. But this test was found least effective in distinguishing 12 varieties from each other. Singh and Afria (1990) reported the GA_3 enhanced seedling growth, emergence and establishment in cotton; Agrawal and Pawar (1990) in soybean, Nagapadma *et al.* (1996) in maize, Walker *et al.* (1992) in sorghum, Shinde and Bhalerao (1990) in sorghum.

Based on these results it is concluded that the chemical tests such as phenol test, $FeSO_4$ test, KOH-bleach test, treatments with growth regulator were found to be useful in classifying the sorghum cultivars. The order of reliability to classify the above 12 sorghum varieties on the basis of chemical tests, KOH test showed marked differentiation followed by the phenol test, modified phenol test and growth response to GA_3. This information would be useful to the plant breeders, seed testing laboratories and seed certification agencies.

Table 2. Distinguishing sorghum varieties on the basis of seedling characters

Varieties	Control		Growth response to GA_3	
	Hypocotyl length	Radicle length	Plumule length	Radicle length
HC-136	Medium	Medium	High	Nil
HC-171	Short	Long	Low	Nil
HC-260	Short	Long	Medium	Nil
HC-308	Short	Long	High	Nil
PC-1	Short	Long	Nil	Nil
PC-6	Long	Medium	Medium	Low
PC-9	Short	Short	Low	Low
PC-23	Short	Short	Medium	Low
PC-121	Short	Long	High	Nil
CSV-15	Long	Long	Low	Nil
SSG59-3	Long	Short	Medium	High
MP-chari	Long	Short	Low	Low

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